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5370

Immunity of the White Rat to Superinfestation with Cysticercus Fasciolaris.

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The only previous report of acquired immunity to cestodes is that of *Taenia solium;* Brumpt[1] states that its presence in the intestine of man prevents added infestation. The experiments here reported demonstrate that one or more larval tapeworms in an advanced stage of development in the liver of the white rat confer a degree of immunity to subsequent infestation. The data are from 2 sources: incidental cases from experiments in which artificial immunity to *C. fasciolaris* was demonstrated,[2] and experiments designed to test acquired immunity directly.

During 4 years' work we have found from time to time rats which harbored one or more huge cysts, as a result of accidental infestation before purchase or in the laboratory. These were discovered at autopsy, 42 days after stock rats, used as controls to artificially immunized rats, were fed by stomach tube with equal amounts of a uniform suspension of onchospheres. Large numbers (21 to 370) of developing larvae (usually 5 mm. in diameter) were found in the liver of control rats, except in the occasional one which had been accidentally infested some months before. In

[1] Brumpt, E., (text-book) 1922 426.

[2] Miller, H. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 926.

these only one huge cyst was present, with small numbers (0-31) of minute dead ones in some cases.

Two lots of rats were experimentally infested on June 6, 1930. Half were again fed onchospheres on October 23 and half on November 7; in each case control rats received equal numbers of onchospheres. Four animals from each lot, and the same number of controls, were autopsied 42 days later, on December 4 and December 18. The remaining animals were reserved for other purposes. The autopsy findings show that the eggs of the second feeding developed in the uninfested control rats and were entirely inhibited in the rats containing 6 months old cysts.

	CONTROLS	PREVIOUSLY INFESTED RATS	
	Average No. of Cysts	Average No.	
		6 Month Cysts	Minute Dead Cysts
Lot 1	113	22	0.25
Lot 2	124	24	0.75

It may be concluded, therefore, that infestation with *Cysticercus fasciolaris,* of from 3 to 6 months' standing, protects the rat host against superinfestation. Experiments are under way to determine whether very large feedings of eggs will override the protection; whether a few very old cysts will furnish as much protection as a large number of younger ones; whether the immunity will disappear, and at what time, after the worms are removed from the huge cysts.

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Observations on the Formation of Wheals. IV. Influence of Calcium Concentrations upon Histamine Wheals.

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We[1] reported that an unidentified substance could be extracted from the skin of dogs which is capable of augmenting histamine wheals. We have now identified this substance as calcium in a certain zone of dilution and only within this zone, as follows:

The proteins in the water extract were precipitated by alcohol. The alcoholic filtrate was evaporated and the residue extracted with

[1] Weaver, W. K., McConnell, F. H., and Alexander, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 486.

water. The active substance was precipitated from this solution by mercuric acetate. It was redissolved in fresh water when hydrogen sulphide was passed through the mixture. The active principle in solution was further separated from organic impurities, phosphates, etc., by treatment with mercuric sulphate and barium carbonate according to the method of West and others. It was found in the filtrate after this process. When this solution was dialyzed against pure water in a thick collodion sac, the active principle did not pass through, but when redialyzed in a thin collodion sac it was recovered from the dialysate. Advantage was taken of this fact to separate the active principle from practically all remaining impurities. Evaporation of the solution after these steps revealed a few crystals which, although small, were suggestive of calcium sulphate. These resisted ashing temperatures although an earlier ashing of an impure fraction had caused the activity to disappear.

With attention focused upon calcium sulphate, experiments were tried in which this pure salt in very weak concentrations was used in place of skin extract. This, too, was found to have an augmenting effect on histamine wheals. Other calcium salts had a similar effect. An interesting similarity in the behavior of calcium sulphate and skin extract was observed. When skin extract was added to histamine solutions it was noted that the size of the wheals was not in proportion to the concentration of the extract. Often one to 10 dilutions of the skin extract produced a larger augmentation than was obtained with the normal concentration, whereas, concentrated extracts often produced less augmentation.

With the discovery of the augmenting effect of calcium sulphate, it was found that the same peculiar behavior was encountered with this salt. It gave the best augmenting effect in low concentrations; frequently only 2 parts per million. The effect diminished upon concentration and finally disappeared entirely. Calcium determinations upon the skin extract then showed that their calcium content fell within the optimum zone found for calcium salts.

Other similarities between the behavior of skin extract and calcium sulphate were observed. It was found that the augmenting power of skin extract disappeared entirely in the presence of excess calcium sulphate, soap, citrates, and when tested on a dog in which acidosis had been induced by means of ammonium chloride. These same substances were found to have a similar inhibiting effect upon the augmenting power of calcium sulphate.

The difficulties experienced in the isolation and identification of calcium as the substance in skin extracts capable of augmenting

histamine wheals have been increased by the fact that many of the reagents employed have contained calcium impurities. Washed ashless filter papers were finally used instead of the ordinary type which contained considerable calcium. Also, silica dishes were employed for the evaporation and ashing of the material.

Other inorganic substances were investigated in regard to their augmenting effect on histamine wheals. It is suspected that magnesium and phosphates have some augmenting power, although the possibility of the existence of calcium impurities in these substances has not been excluded. Other tissues besides skin were found to give active extracts. Among these was blood which was used as the most common source since it furnished a solution as a starting point.

Control tests were run on all solutions tested in order to make sure that there was not enough histamine or calcium in the extracts to give wheals in the absence of added histamine.

Atropine and codeine, which also are wheal-forming substances, are likewise augmented both by skin extracts and by calcium.

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Response of Intact Small Intestine in Non-anesthetized Dogs to Cathartic Agents, to Morphine and Atropine.

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Many earlier investigators used cathartic drugs subcutaneously and intravenously, but due to the toxicity of and local irritation caused by these methods of administration they were soon discontinued. Podwyssotzki[1] administered subcutaneously an extract of podophyllin and observed that catharsis resulted. His results were confirmed by Neuberger[2] and Dixon.[3] Dixon also reported catharsis by subcutaneous injections of podophyllotoxin. Craig[4] reported catharsis from extract of aloe. His results were later contradicted

[1] Podwyssotzki, *Schmiedeberg's Arch.*, 1880, **13**, 29.

[2] Neuberger, *Schmiedeberg's Arch.*, 1891, **28**, 32.

[3] Dixon, *Therap. Monatshefte*, 1903, **16**, 102; *Brit. Med. J.*, 1902, **18**, 1244.

[4] Craig, *Verchow-Hirsch. Jahresber.*, 1875, **1**, 493.

by Cohn[5] but confirmed by Hiller,[6] Meyer[7] and Kohlstock.[8] The latter experimenters used aloin in aqueous solution subcutaneously. That the extract of colocynth caused catharsis when injected subcutaneously was demonstrated by Radziejewski[9] and confirmed by Hiller,[6] Cascara sagrada was administered subcutaneously by MacCallum[10] and he observed a cathartic action. His results were confirmed by Ott and Scott[11] who used extract of cascara and by Brauer[12] and Pietch[13] who used the water soluble glucoside of cascara sagrada "peristaltin". Both Chistoni[14] and Walther[15] were unable to produce catharsis by the subcutaneous injection of the aqueous extract of this drug. The fact that phenolphthalein causes catharsis when administered either subcutaneously or intravenously was shown by Ott and Scott,[11] and Abel and Rowntree.[16] Ott and Scott[11] were the only investigators to study the effect of these drugs on the contractions of the intestine. They observed the fact that both phenolphthalein and cascara sagrada increased the height of the contractions with a loss of the general tonus.

This investigation was undertaken to shed some light upon the mode of action of these drugs on the intestine in producing purgation.

Non-anesthetized dogs with Thiry-Vella fistulae were employed. Only the ileum was studied. A rubber balloon, connected to recording Brodie Bellows, was inserted in the lumen of the gut to write the changes in general tonus and the contractions. Phenolphthalein, bitter and bitterless glucosides of cascara sagrada (P-D and Co.), extract of rhubarb, extract of podophyllin and the extract of aloe were employed. In some instances, the effects of atropine sulphate in 3 to 5 mg. doses and morphine sulphate in 1 to 10 mg. doses, upon the changes produced by the cathartic drugs were also studied. The drugs were administered either intravenously, or injected into the lumen of the fistula. Morphine also was given by mouth in small doses.

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[5] Cohn, *Berlin. Klin. Wochenschr.*, 1882, **68**.

[6] Hiller, *Z. f. Klin. Med.*, 1882, **4**, 481.

[7] Meyer, *Schmiedeberg's Arch.*, 1891, **28**, 186.

[8] Kohlstock, *Chorité Annalen*, 1892, **17**, 283.

[9] Radziejewski, *Arch. Anat. u. Physiol.*, 1870, **1**.

[10] MacCallum, *Univ. Calif. Publications Physiol.*, 1903-04, **1**, 163.

[11] Ott and Scott, *Med. Bull. Phila.*, 1908, **30**, 90. *Ott's Contribution to Physiol.*, 1909, Nos. 7 and 18.

[12] Brauer, *Münch. Med. Wochenschr.*, 1910, 1812.

[13] Pietch, *Therap. Monatsheft.*, 1910, **24**, 35.

[14] Christoni, *Arch. Farm.*, 1914, **17**, 97.

[15] Walther, *Münch. Med. Wochenschr.*, 1910, 1812.

[16] Abel and Rowntree, *J. Pharm. Exp. Therap.*, 1919, **1**, 231.

The usual reaction of the intestinal loop to the cathartic drug when injected intravenously was a sudden decrease in the general tonus and a decrease in the height of the rhythmical contractions. If peristaltic contractions were present these were temporarily inhibited. Peristaltic contractions usually began, without changing the decreased general tonus, 2 to 10 minutes after injection of the drug. The amplitude of these contractions increased gradually until in many instances their severity caused such pain that the animal became restless and whined during each contraction. Borborygmi were always heard. When the cathartic was placed in the lumen of the gut the first response was a rise in general tonus. This was followed by a decrease in general tonus to a point below the control level and by the introduction of peristaltic contractions.

Atropine when injected at the height of the cathartic action always temporarily produced a decrease in general tone and a disappearance of the peristaltic contractions. Although the rhythmical contractions continued they were always decreased in amplitude. Following this sudden change in tonus and activity of the gut the peristaltic contractions reappeared, but were less extensive and of longer duration than before, more nearly the type normally seen in the unmedicated gut.

Morphine sulphate when injected intravenously, when injected into the lumen of the gut, or when given by mouth invariably caused a sudden increase in the general tonus of the gut and a temporary disappearance of the peristaltic contractions.

5373

Utilization of Carbon Compounds by Mycobacteria in a Synthetic Medium.

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The utilization of a number of carbohydrates, when added to plain broth as the basic medium, by organisms of the genus Mycobacterium was reported previously.[1] The method employed was limited to determinations of utilization of reducing sugars only. In order to determine the extent to which other carbon compounds were

[1] Merrill, Malcolm H., *J. Bact.*, 1930, **20**, 235.

utilized, a synthetic medium has been used which contained the carbon compound under investigation as the sole carbon source. The appearance of growth and reaction change in the media were used as criteria for utilization.

Employing this method the extent of utilization of 16 carbon compounds by 14 strains of Mycobacteria has been studied. The

TABLE I.

Summary of results on utilization determinations.

	Myco. tuberculosis H-37	*Myco. tuberculosis* M-1	*Myco. tuberculosis saprophytic*	*Myco. tuberculosis bovis*	*Myco. leprae 282*	*Myco. avium*	*Myco. ranae*	*Myco. chelonei*	*Myco. smegmatis*	*Myco. butyricum*	*Myco. berolinensis*	*Myco. fribburgensis*	*Myco. stercusis*	*Myco. phlei*
Glucose	±	+	+	±	±	±	+	+	+	+	+	+	+	+
Fructose	o	o	+	o	+	o	o	+	+	+	+	+	+	+
Galactose	o	o	+	o	o	o	o	o	o	±	+	±	+	+
Arabinose	o	o	o	o	+	o	+	o	o	o	+	o	o	o
Maltose	o	o	+	o	o	o	+	o	o	o	+	o	o	o
Lactose	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Sucrose	o	o	+	o	+	+	+	+	+	+	+	+	+	+
Raffinose	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Inulin	o	o	o	o	o	o	o	o	o	o	o	±	+	o
Mannitol	o	o	+	o	+	+	±	+	+	±	+	+	+	+
Glycerol	o	o	+	o	o	+	o	o	o	o	+	o	+	+
Ethyl alcohol	o	o	o	o	o	o	±	o	o	o	+	o	+	+
Salacin	o	o	o	o	o	o	o	o	o	o	+	±	+	+
Sodium lactate	+	+	+	+	+	±	+	+	+	+	+	+	+	+
" citrate	o	o	o	o	o	o	o	o	o	o	o	o	o	o
" acetate	o	o	o	o	o	o	o	o	o	o	o	o	o	o

The symbols have the following meaning: + indicates definite utilization; ± indicates slow utilization; 0 indicates no evidence of utilization.

results are summarized in Table I. The utilization is sufficiently varied that it becomes of some value in differentiating the organisms studied. There were some cases in which utilization could not be demonstrated by this method where the same carbohydrates were shown to be utilized by the same organisms in the carbohydrate broth cultures.[1]

The reaction changes associated with growth of the organisms in the synthetic media were progressive increase in acidity in the carbohydrate and alcohol containing media, and a primary increase in alkalinity followed by a reversion of the reaction towards neutrality of the media containing organic acids.

New York Section.

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5374

Injections of Cortin on Resistance of Suprarenalectomized Rats. Biological Assay of Extracts of Suprarenal Cortex.

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(With the cooperation of F. A. Hartman.)

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The resistance of suprarenalectomized rats to toxins, poisons, bacterial and protozoan infections is markedly depressed.[1, 2, 3, 4, 5] The height of susceptibility is reached at the end of the first week following bilateral suprarenalectomy. Suprarenalectomized rats succumb to small doses of typhoid vaccine.[4] In previous experiments typhoid vaccine has been used as a gauge of suprarenal insufficiency.

In the experiments here reported the protective effect of an extract of the cortex of the suprarenal gland prepared by us according to the method of F. A. Hartman[6] was determined.

Three months old adult suprarenalectomized rats were used. Injections of a highly concentrated extract* of cortex were administered twice daily intraperitoneally. The M.L.D. for suprarenalectomized rats of a batch of typhoid vaccine was determined. The experiment was divided into 4 groups. One group of 17 rats received cortical extract twice daily from the day of operation to the end of the experiment. Of these, 4 rats were injected on the sixth day with one M.L.D., 5 were injected with 2 M.L.D., 4 with 4

[1] Scott, W. J. M., *J. Exp. Med.*, 1923, **38**, 543.

[2] Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 506.

[3] Belding, D., and Wyman, L. C., *Am. J. Physiol.*, 1926, **38**, 50.

[4] Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

[5] Marmorston-Gottesman, J., Perla, David, and Vorzimer, Jefferson, *J. Exp. Med.*, 1930, **52**, 587.

[6] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

* 1 cc. equivalent to 40 gm. of cortex. The rats received 20-40 gm. of cortex a day.

M.L.D., and 4 with 6 M.L.D. of typhoid vaccine. A second group of 10 rats received equivalent amounts of salt solution. These were injected on the sixth day with one M.L.D. A third group of 8 rats was untreated and on the sixth day after operation these were injected with one M.L.D of typhoid vaccine. A fourth group of 4 rats received cortical extract during only the 24-hour period before and after the injection of one M.L.D. of typhoid vaccine on the sixth day.

Results: The rats of Group 1 that had been repeatedly injected with cortical extract survived as much as 4 M.L.D of typhoid vaccine. The rats injected with 6 M.L.D. died and one with 4 M.L.D died. The rats of Group 4 that had received cortical extract only during the last 24-hour period survived one M.L.D of typhoid vaccine. Of the 18 rats of Groups 2 and 3 that had received no cortical extract or had been injected with salt solution all but 2† were killed by one M.L.D. of typhoid vaccine. Most of the rats in these 2 groups lost from 10 to 40% of their body weight during the first week after operation. Several showed some degree of clinical insufficiency and one rat died during the week. All the rats that had been repeatedly injected with cortical extract equalled or surpassed their preoperative weight by the sixth day after the operation.

These experiments indicate that the cortical extract of Hartman raises the resistance of suprarenalectomized rats.

Hartman[7] suggested the maintenance of the weight curve in immature rats as a means of assaying the potency of his extract. Though the maintenance of the weight curve is an evidence of the activity of an extract, it cannot be utilized as a quantitative biological assay of potency. We believe that the effect of an extract of cortex on the resistance of suprarenalectomized rats to typhoid vaccine may be used as a biological assay of the potency of such extracts. A standard rat unit may thus be readily established for each batch of cortical extract. Since those rats treated during the last 24 hours prior to the injection of typhoid vaccine show increased resistance to typhoid vaccine, a rat unit of cortical extract may be established as that quantity of extract necessary to raise the resistance of suprarenalectomized rats sufficiently to survive the M.L.D. of typhoid vaccine injected on the sixth day following the operation.

Four rats were injected with the extract of 80 grams of cortex in

† Both these rats gained steadily in weight and had large accessories.

[7] Hartman, F. A., and Thorn, G. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 94.

divided doses only during the 24-hour period before and after the injection of one M.L.D. of typhoid vaccine administered on the sixth day after suprarenalectomy. Two rats received 40 grams of cortex in the same period and 2 rats received 30 grams of cortex in this interval. The rats that had received 30 grams were killed by one M.L.D. of typhoid vaccine, those receiving larger amounts survived. In this experiment, therefore, 1 minimal protecting rat unit (1 M.P.D.) is equivalent to 40 grams of cortex.

The effect of larger amounts of cortin administered during the last 24-hour period before and after the injection of typhoid vaccine was determined. Six suprarenalectomized adult rats were injected with 200 grams of cortex during the 24-hour period on the sixth day after suprarenalectomy. At the time the first injection was given the rats all lost 10-25% of their preoperative weight. Three of the rats received 4 M.L.D. of typhoid vaccine and 3 received 2 M.L.D. Two rats in each group survived. Three control saline treated rats were killed with 1 M.L.D. of typhoid vaccine. The effect on the resistance of suprarenalectomized rats of injections of cortin is marked in spite of the loss of weight the animal had sustained prior to the administration of cortin.

The effect of a single large dose of cortin administered immediately after suprarenalectomy on the resistance of adult rats to 1 M.L.D of typhoid vaccine administered on the sixth day after the operation was studied. Three rats received 160 gm. of cortex (4 cc. of extract) intraperitoneally in a single injection soon after suprarenalectomy. Two rats received the same quantity of saline. The weight of the treated rats was maintained during the entire week. The controls lost 12 and 15% of the preoperative weight. One of the three treated rats was killed with 1 M.L.D. as well as the controls. Though a single injection of a large amount of cortin may maintain the weight curve for a week it will not raise the resistance as high as repeated injections of small amounts of cortin.

Preliminary experiments on the effects of the injection of cortin on the resistance of suprarenalectomized rats to histamine have been made. Suprarenalectomized rats treated with cortin will resist at least three minimal lethal doses of histamine (ergotamine acid phosphate).[8] The details of these experiments will be reported in a subsequent publication.

[8] Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

5375

Protection of Adrenalectomized Animals Against Bacterial Intoxication.

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In 1924, Scott[1] first showed the greatly reduced resistance of animals with latent adrenal insufficiency to intoxication by pyogenic bacteria. Numerous attempts to protect with cortical extract against this fatal effect were carried out unsuccessfully, the difficulty being to get rid of epinephrin without destroying the active cortical hormone. Recently Scott and Bradford[2] found some increased resistance to bacterial intoxication conferred by the administration of a cortical extract (Swingle and Pfiffner[3]). A difficulty in this experiment was that no satisfactory criterion of adequate substitution was available. In the present work the growth curve is used as an indication of adequate dosage of the cortical hormone.[4] As the function of the adrenal cortex to protect against the harmful effects of bacterial intoxication is important in clinical medicine, we have tried to prove beyond any doubt that an extract of the adrenal cortex offers such protection.

The growth curves of 32 young adult male rats were determined for 2 weeks. Both adrenals were then removed at one operation. The animals were divided into 2 groups of 16 each. All were injected with the same volume of fluid and were weighed daily. The animals of Group A were injected with cortin obtained by the ether-alcohol method,[5] those of Group B with isotonic saline. The extract used in the first week was prepared by a new method which was found to greatly reduce its potency. Two rats in each group died in this interval. A potent extract was used after the first week, under the influence of which the weight curves of the extract-injected animals recovered their preoperative slope. The product from 25 gm. of cortex was injected twice daily in each rat

[1] Scott, W. J. M., *J. Exp. Med.*, 1924, **39**, 457.

[2] Scott, W. J. M., and Bradford, W. L., PROC. SOC. EXP. BIOL. AND MED., in press.

[3] Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 321.

[4] Hartman, F. A., Brownell, K. A., and Hartman, W. E., *Am. J. Physiol.*, 1930, **95**, 670.

[5] Hartman, F. A., and Thorn, G. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 94.

in Group A. In order to increase the protection at the time it was most needed, the injections were made more frequently during the 3 days before the test with killed bacteria; at first 3 times daily and in the final 24 hours 5 times, the controls receiving saline injections of the same volumes and frequencies. Two weeks after adrenalectomy, each animal of both groups was given 1½ cc. of standard typhoid vaccine (one billion killed organisms per cc.) intraperitoneally. Subsequent to this the animals were injected with extract and saline respectively about every 2 hours for 10 hours. Six hours after the injection of bacteria all the animals in Group B (saline injected) were dead and none of those in Group A (cortin injected). Four of the rats in Group A subsequently died during that night. Ten of them (71%) survived indefinitely

The resistance of adrenalectomized rats to bacterial intoxication had been significantly increased by an extract of the adrenal cortex.

5376

Effect of Nutritional Anemia on Size of the Heart.

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During an investigation pertaining to the influence of certain foods on anemia in rats, it was observed that the hearts of those having low hemoglobin values were considerably larger than the hearts of normal animals. Thereupon the weights of the hearts of the experimental animals, computed on the percentage basis, were compared with those of normal animals as given by Donaldson. Successive biweekly hemoglobin determinations were made throughout the period of study, the last being taken just before the animals were killed. The results indicate that there is a close correlation between the size of the heart and the degree of anemia. The heart weights of animals with high hemoglobin values—from 11 gm. to 14 gm. per 100 cc. of blood (Newcomer method) are comparable to those given by Donaldson. When the hemoglobin values fell to 10 gm. the hearts were slightly hypertrophied; this became more marked as the degree of anemia increased. At the very low hemoglobin levels, from 2 gm. to 3 gm. per 100 cc., the heart weights averaged approximately 3 times that of normal animals.

TABLE I.

Relation of Heart Weight to Hemoglobin of Rats Receiving Milk Diets with the Addition of Various Substances Used in the Artificial Feeding Mixtures of Infants.

Diet	No. Animals	Time on diet	Body Weight aver.	Heart Weight		Hemoglobin per 100 cc. blood
				Exper. Animal	Normal Animal	
		weeks	gm.	gm.	gm.	gm.
Pasteurized milk	1	13	172	1.3	0.71	3.61
Milk-tomato juice	3	10	106	1.3	0.49	2.71
" -orange juice	3	10	86	1.14	0.42	2.34
" -wheat embryo extract	3	6	99	1.07	0.47	4.02
" -Dextri-Maltose No. 2	3	13	224	1.46	0.87	5.15
" -autolyzed yeast	3	12	186	1.04	0.77	6.87
" -Mellin's Food	2	18	279	0.97	1.04	11.90
" -corn syrup	3	17	243	1.10	0.90	10.33
" -Dextri-Maltose with Vitamin B	2	15	245	0.90	0.93	10.69
" -Vitavose	2	18	239	0.94	0.92	11.95
" -Malto-Dextrin with added Fe and Cu	2	12	198	0.77	0.79	12.70
" -egg yolk dried (2.8 gm.)	2	6	169	0.83	0.74	11.29
" -liver (0.4 per day)	2	6	150	0.64	0.64	11.48
" -liver *ad lib.*	2	6	199	0.78	0.80	14.60
" -Vitavose with added Fe and Cu	3	10	198	0.88	0.79	13.32

5377

The Bactericidal Power of Viosterol.

B. E. MONTGOMERY. (Introduced by C. I. Reed.)

From the University of Illinois College of Medicine.

In an effort to determine whether irradiated ergosterol (viosterol) is capable of exerting any direct bactericidal power the following experiments were performed. Since the material used was in solution in corn oil, control experiments with pure corn oil were made for comparison. The preparation used was superacterol (1000D),* 10 mg. ergosterol per cc.

Agar tubes were melted and cooled to 50°C. Mixtures were made as follows:

1 cc. viosterol	—10 cc. agar	1 cc. corn oil	—10 cc. agar
2 " "	—10 " "	2 " " "	—10 " "
3 " "	—10 " "	3 " " "	—10 " "
4 " "	—10 " "	4 " " "	—10 " "
5 " "	—10 " "	5 " " "	—10 " "

These tubes were shaken, poured into sterile petri dishes and cooled. The resulting mixture was a milky emulsion of the oil in the agar. One loopful of a 24-hour broth culture of *B. coli* was planted on the surface of each plate. Plain agar plates seeded in the same manner were used as controls. The plates were incubated 24 hours at 37.5°C. The growth on all the plates presented the same macroscopic characteristics. There was no evidence of inhibition of growth nor any modification of colony formation.

Flasks of broth prepared in the same manner and with identical proportions incubated for 24 hours at 37.5°C. were also negative as to inhibition of bacterial growth.

Likewise samples taken from these flasks and inoculated into plain agar plates showed no differences between the controls and the viosterol growths or characteristics.

A duplicate set of experiments with *Staphylococcus aureus* were also negative.

Conclusion: Neither irradiated ergosterol in corn oil, nor pure corn oil itself, inhibits the growth of cultures of *B. coli* or *Staphylococcus aureus* nor modifies the cultural characteristics of these organisms.

* This material was obtained through the courtesy of Dr. C. E. Bills, Mead, Johnson & Co.

5378

Galvanometer Tracings Obtained in a Physico-Chemical Schema Simulating an Electrocardiogram, and the Influence of Membranes on These Records.

JANE SANDS ROBB. (Introduced by M. S. Dooley.)

From the Department of Pharmacology, Syracuse University.

A schema is described in which interruptions of a direct current of known direction and intensity in the primary circuit are recorded on a string galvanometer which forms part of the secondary circuit. Evidence was presented to show that the apparatus was adequate for the problem concerned. When the nonpolarizable electrodes dip into the open bowl, or when the primary electrodes are enclosed in a collodion membrane, making and breaking of the primary circuit results in a single monophasic deflection, the string maintaining a constant position as long as the key is closed. If the

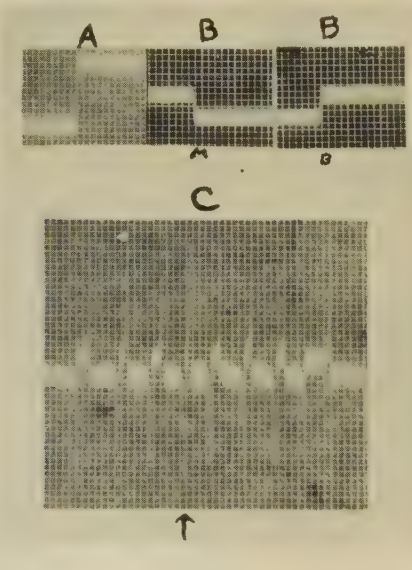

FIG. 1.

A. Standardization: 1 cm. = 1 mv.

B. Galvanometer response, when primary electrodes are enclosed in a collodion membrane, and when the primary circuit is interrupted. Interval deleted between make make and break = 10 seconds.

C. Galvanometer response when primary electrodes are enclosed in a protein coated collodion membrane. Note the appearance of "Quick" deflections upon making and breaking of the primary circuit. If the interval between make and break is very brief, the record simulates the Q-R-S group of the electrocardiogram (indicated by arrow in C).

primary electrodes are enclosed in a gelatine coated membrane, a quick deflection occurs when the primary circuit is made and again when it is broken. If the make and break recur rapidly, these quick deflections simulate the QRS of an electrocardiogram. The importance of membrane polarization as a factor causing distortion of electrocardiograms is stressed. A full report is in press.[1]

5379

Specific Dynamic Action of Meat, Glycine, and of Meat Plus Glycine in Man.

E. S. NASSET, T. B. GARLICK AND R. W. SWIFT.
(Introduced by J. R. Murlin.)

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Preliminary results on 2 human subjects indicate that the increase in metabolism caused by the ingestion of 300 gm. of lean meat is approximately double that resulting from the ingestion of 150 gm. Twenty-five gm. of glycine, equivalent to 150 gm. of meat as regards nitrogen content was taken by one subject, and 50 gm. of glycine, corresponding to 300 gm. of meat, was taken by the other, both alone and with 300 gm. of meat. In one subject the maximum metabolism observed after ingestion of 25 gm. of glycine and after ingestion of 150 gm. of meat agreed rather closely. The effect of 50 gm. of glycine on another subject, however, caused a smaller increase than 300 gm. of meat.

No summation of effect was obtained when 50 gm. of glycine was ingested an hour and a quarter after 300 gm. of meat. In the case of one subject, the effect of glycine alone was without marked irregularity, while in the other marked irregularity was shown, though not as great as has been reported by others in experiments on the dog.[1] As has been noted by other workers,[2, 3] the urinary nitrogen excretion is probably not a very exact measure of the protein katabolism over a short period.

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[1] 1930 Year Book of the Physicians Hospital of Plattsburgh, N. Y.

[1] Weis, R., and Rapport, I., *J. Biol. Chem.*, 1924, **60**, 513.

[2] Gephart, F. C., and DuBois, E. F., *Arch. Int. Med.*, 1915, **15**, 835.

[3] Williams, H. B., Riche, J. A., and Lusk, G., *J. Biol. Chem.*, 1912, **12**, 349.

5380

Effects of the Halogenation of Oxyquinoline on Biological Activity.*

HAMILTON H. ANDERSON, NORMAN A. DAVID AND DOROTHY A. KOCH.
(Introduced by C. D. Leake.)

From the Pharmacological Laboratory, University of California Medical School, and the Pacific Institute of Tropical Medicine, Hooper Foundation for Medical Research, San Francisco.

Chiniofon, N. N. R., introduced commercially as "yatren", and first tried clinically in amebiasis by Mühlens and Menk[1] in 1921, has had some popularity as an amebicidal agent. Chemically chiniofon is sodium-iodoxy-quinoline-sulphonate, and is related to chinosol N. N. R. (oxyquinoline sulphate) and vioform N. N. R. (iodochloroxyquinoline). Since chiniofon has been claimed to have amebicidal activity, we thought it might be of interest to study it from this standpoint in comparison with as many related compounds as we could secure. Such an effort we thought might yield significant information regarding the relation of chemical constitution to

TABLE I.
Summary of some Data Relating to Toxicity, Balantidicidal Action, and Amebicidal Concentration of Certain Halogenated Oxyquinoline Derivatives.

Drug.	Proportion of Deaths and Oral Lethal Dose in Guinea Pigs	Oral Balantidicidal Dose in Guinea Pigs	Amebicidal Concentration *in vitro* 24 hours
Oxyquinoline	2/10 at 1200 mgm./Kg.	20% cured at 1250 mgm./Kg.	Not tested, insoluble in water
Oxyquinoline sulphate	1/10 at 1200 " "	No cures at 1250 mgm./Kg.	1:10,000
Chloroxyquinoline	5/15 at 1200 " "	Not tested	1:10,000†
Na-Iodoxyquinoline sulphonate	7/15 at 900 " "	60% cured at* 600 mgm./Kg.	1:500
Iodochloroxyquinoline	7/10 at 200 " "	80% cured at* 150 mgm./Kg.	Not tested, insoluble
Diethyl-amino-dimethylene-hydroxy-iodochlorquinoline HCl	5/10 at 250 " "	Not tested	1:50,000

* 20% mortality at this dose.
† In 25% buffered ethylene glycol, which has no amebicidal action in itself.

* This report is based on part of an extended cooperative study of amebiasis, supported in part by Eli Lilly and Co., Indianapolis, and the Ciba Co., Inc., New York City.

[1] Mühlens, P., and Menk, W., *Munch. Med. Wochnschr.*, 1921, **68**, 802.

pharmacological action in this series of oxyquinoline derivatives, particularly with reference to the effects on biological activity of introducing various halogens into the oxyquinoline molecule.

We secured the following compounds for this investigation: 1. Oxyquinoline, from Dr. J. V. Barrow. 2. Oxyquinoline sulphate (Chinosol, N. N. R.). 3. Chloroxyquinoline, from the Ciba Co., Inc. 4. Sodium-iodoxyquinoline sulphonate (Chiniofon, N. N. R.). 5. Iodochloroxyquinoline (Vioform—Ciba, N. N. R.). 6. Diethylamino-dimethylene-hydroxy-iodochlorquinoline HCl, from the Ciba Co., Inc.

In this series, compounds 3 and 4 are monohalogenated derivatives of oxyquinoline, while in compounds 5 and 6, two different halogen atoms have been placed in the oxyquinoline molecules.

These substances have been studied with respect to toxicity on oral administration to guinea-pigs, rabbits, and cats; balanticidal action in naturally infested guinea pigs; amebicidal action *in vitro,* and therapeutic effect in monkeys naturally infested with intestinal parasites. The techniques followed have been previously described.[2] A general summary of part of this study appears in Table I.

It may be noted that toxicity increases with halogenation of oxyquinoline, and in proportion to the atomic weight of the halogen. Thus chloroxyquinoline is slightly more toxic than oxyquinoline, and the iodoxyquinoline compound is slightly more toxic than the one containing chlorine. The addition of both iodine and chlorine to oxyquinoline results in a considerable increase in toxicity, but the further addition of a solubilizing group reduces the toxicity somewhat. Similarly balanticidal action in naturally infested guinea pigs seems to increase with increasing halogenation of oxyquinoline. But amebicidal action *in vitro* does not seem to be related in this same way to the chemical constitution of this series of drugs.

In monkeys naturally infested with *Endameba histolytica,* 900 mgm./Kg. of iodochloroxyquinoline was tolerated in divided doses over a 6 weeks period with eradication of amebae during the time of observation. Further studies are indicated in order to evaluate the usefulness of these compounds in treating protozoan infestation in mammals.

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[2] Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 267; Leake, C. D., Koch, D. A., and Anderson, H. H., *Ibid.,* 1930, **27**, 717; *Am. J. Trop. Med.,* 1930, **10**, 249; David, N. A., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 196.

5381

A New Method for the Determination of Allantoin in Dog's Urine.

FRANK W. ALLEN AND LEOPOLD R. CERECEDO.
(Introduced by Carl L. A. Schmidt.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

Fosse and Bossuyt[1] have described a new method for the determination of allantoin. In their procedure allantoin is first hydrolyzed by alkali to allantoic acid. In a second step the allantoic acid is broken down by acid hydrolysis to urea and glyoxylic acid. By estimating colorimetrically the amount of glyoxylic acid present, Fosse and Bossuyt calculate the quantity of allantoin originally present in the sample analyzed.

The technique of Fosse and Bossuyt in our hands did not yield satisfactory results. Besides, we preferred to determine urea rather than glyoxylic acid as an index of the amount of allantoin present in urine.

The new procedure is as follows: To 10 cc. of diluted dog's urine 5 cc. of a 10% solution of urease (Squibb) are added. The mixture is incubated at 38° for 1½ hours. The protein is then precipitated with Tanret's Reagent and filtered off. An aliquot part of the filtrate is neutralized with N KOH using phenolphthalein as indicator, and an excess of alkali added so as to obtain a final concentration of 0.2 N KOH. The mixture is kept for 2 hours at 70°. It is then neutralized with N HCl, and an excess of acid added until the acidity of the solution is 0.1 N. Warming at 70° for 30 minutes hydrolyzes the allantoic acid present to urea. From the amount of urea present, estimated according to the method of Allen and Luck[2], the quantity of allantoin originally present in the sample can be calculated.

By this procedure we are able to estimate added amounts of allantoin in dog's urine in yields better than 97%. The quantity of allantoin excreted by dogs, weighing from 8 to 20 kg. in 24 hours ranges between 200-1400 mg., depending upon the total nitrogen per diem.

[1] Fosse, R., and Bossuyt, *Compt. rend. Acad.*, 1929, **188**, 107.
[2] Allen, F. W., and Luck, J. M., *J. Biol. Chem.*, 1929, **82**, 693.

5382

Blood Sugar Response to Intravenous Insulin in Normals and in Diabetics.

WILLIAM S. COLLENS AND HAROLD G. GRAYZEL.

From the Pediatric Research Laboratories, Jewish Hospital of Brooklyn, N. Y.

Studies in the literature lead one to conclude that the blood of diabetics contains a substance that has an inactivating influence upon insulin. Some observers have been led to believe that this phenomenon plays a rôle in the pathogenesis of diabetes mellitus. Karelitz[1] studied the effect of insulin after its incubation with normal and diabetic serum and found that diabetic serum has a definite retarding influence upon the activity of insulin. He concluded that diabetic serum contained an anti-insulin substance. Loewi[2, 3] has recently withdrawn his previous hypothesis that the liver elaborates a substance that is capable of inactivating insulin. He called this substance glykamin. Epstein[4] believed that the presence of trypsin in the blood inactivates insulin and thought that this is the cause of diabetes.

On the basis of such conclusions, one may reasonably suspect that equivalent intravenous doses of insulin would show a less marked depression in blood sugar in the diabetic than in the normal individual. We, therefore, undertook a study of the comparative response of the blood sugar of the normal and diabetic subjects to the intravenous administration of insulin.

Method. The intravenous route was used in order to eliminate the factor of absorption from the subcutaneous tissue. Twelve diabetic patients, encompassing a wide variation in severity and age incidence, were selected for these studies. Two normal human beings and a normal dog were used as controls. The blood sugar content was studied by the Folin-Wu method. Lilly insulin of U 20 strength was employed. 0.2 of a unit of insulin per kgm. body weight was given in all the cases. This dose was selected on the basis of preliminary trials, as the desirable optimal dose for these experiments. The fasting blood sugar was obtained after a 14-hour fast and blood was taken at the following intervals after the intra-

[1] Karelitz, S., Cohen, P., and Leader, S. D., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 11.

[2] Loewi, O., *Wien. Klin. Woch.*, 1926, **39**, 1074.

[3] Loewi, O., *Wien. Klin. Woch.*, 1928, **8**, 391.

[4] Epstein, A. A., *J. Am. Med. Assn.*, 1925, **85**, 29.

TABLE I.

Name	Age Yrs.	Fasting Blood Sugar	Insulin 0.2 U per Kgm.	Blood Sugar After Insulin in Hours mgm. per 100 cc. blood									Total Drop mgm. %	Time of Max. Drop
				¼	½	¾	1	1½	2	2½	3	4	5	
		Normal												hr.
H. G.	30	117	13.0	119	74	89	95	110	111	108	106			43	½
W. C.	32	114	14.0	107	67	82	89	87	109					47	½
Dog		93	3.4	96	46		50	63	90	95	98			47	½
		Diabetic													
S. C.	8	363	5.0	342	236	204	187	141	113		88			275	3
Y. K.	9	151	4.3	149	120	82	83	81	91		85			70	1½
M. G.	12	193	9.0	126	100	80	99	103	104	130				113	¾
A. E.	13	365	5.0	312	262	229	198	131	119	102				253	2½
J. L.	24	303	13.0	295	248	219	197	153	129	104	111			199	2½
B. L.	14	269	7.8	228	147	92	78	85	97	102	106			191	1
M. S.	44	203	12.0	158	136	114	97	100	96	111	107			106	1
M. G.	51	195	15.0	110	65	72	80	90	106	111				130	½
T. K.	25	220	11.0	191	162	116	100	75	70	78	77			152	1½
S. C.	8	398	6.0				195		86		62	66	86	336	2½
M. W.	11	133	9.0	111	102		67	61	65	62	63			72	1½
K. M.	19	278	11.0	263	244	189	166	123	111	78	64			214	3

venous administration of insulin: ¼, ½, ¾, 1, 1½, 2, 2½, 3, 4, and 5 hours.

Results. It will be observed from the accompanying table that

in the three control experiments there was a uniform response to the injection of intravenous insulin. It is noted that there is a 15-minute refractory period, during which time the blood sugar level remained essentially the same as it was before the insulin was administered. At the end of ½ hour, the blood sugar dropped precipitously to its lowest level. Within 45 minutes after the injection, the blood showed evidence of rapid recovery of its sugar content. The total drop in these 3 control experiments was between 43 and 47 mgm. %. In both humans there occurred mild symptoms of hyper-insulinism, which lasted about 5 minutes, from which there was a spontaneous recovery.

It is seen from the table that in most of the diabetic patients there existed no refractory period, that the blood sugar showed evidence of rapid drop 15 minutes after injection. The depression continued for a much longer period than in the normals. In the milder diabetics, the maximum drop occurred from 45 minutes to 1 hour after injection, and in the severest diabetics the blood sugar was still dropping at the end of 3 hours. The total drop in blood sugar ranged between 70 and 336 mgm. % in the various cases, and the higher the initial fasting blood sugar the greater the total drop.

This enormous drop in blood sugar in diabetics of 70 to 336 mgm. %, is far different from the disappearance of an average of 43 to 47 mgm. % in controls, with the same dose of insulin. It seems difficult to reconcile the conclusions drawn from such results, with the possibility of the presence of an inactivating substance in the blood of diabetics. The presence of such a substance should prevent such phenomenal depressions. The nature of this response resembles the results obtained by Thaysen,[5] and Norgaard and Thaysen.[6]

It is interesting to mention that the symptoms of hyper-insulinism occurred with blood sugars of 74 and 67 mgm. % in the normal, and of 75, 78, 65, 61, and 62 mgm. % in the diabetic patients. The blood sugar of one diabetic reached 64 mgm. % without any symptoms. The symptoms existed at the lowest level of the curve and all these cases recovered spontaneously.

Conclusions. Equivalent doses of insulin on the basis of body weight when administered intravenously, produce a much greater depression in the blood sugar level in diabetics than in normals. This seems to indicate the absence of any substance in the blood of diabetics which might inhibit insulin action.

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[5] Thaysen, T. E., Hess, *Hospitalstidende,* 1930, **73**, 357.

[6] Norgaard, A., Thaysen, T. E., Hess, *Hospitalstidende,* 1929, **72**, 881.

5383

Effect of Amytal upon the Fetus and its Transmission Through Placenta of White Rat.

CHARLES M. BOUCEK AND ARTHUR D. RENTON.
(Introduced by J. C. Donaldson.)

From the Department of Anatomy of the University of Pittsburgh.

The widespread use of sodium amytal in obstetrics and in animal experimentation, where observations upon the fetuses are to be made, has necessitated a study of the placental transmission of this anesthetic. A review of the literature shows that there is general agreement that there is no danger to the fetus when the mother is anesthetized with this drug.

Our experiments were divided into 2 main groups: (1) those showing the effect upon the fetuses of anesthesia of the mother with sodium amytal, and (2) experiments showing the effect upon mothers of injections of this same anesthetic into the fetuses. In the first group, pregnant white rats were completely anesthetized with a 2% solution of sodium amytal. The anesthetic dose used was 80 mg. per kilo of body weight minus the estimated weight of the fetuses. It was found that the dose of sodium amytal calculated from the total weight of the pregnant animal proved lethal for the mother in 60% of the cases, but doses calculated from the weight of the mother minus the estimated weight of the fetuses were never lethal. In some cases, small amounts of ether were necessary to supplement the sodium amytal anesthesia. At various intervals, during the period of anesthesia of the mother, the fetuses were tested, both *in utero* and when removed from the uterine cavity. When stimulated with a needle, they responded as did the normal controls. We conclude, therefore, that sodium amytal does not pass the placenta from mother to fetus in large enough quantities to be detected by the gross tests employed.

In the second group of experiments, which were performed to determine the transmission of sodium amytal from fetus to mother, the fetuses or amnia were injected with the minimum amount of the drug necessary for complete anesthesia of the mother. The mothers became anesthetized almost as quickly as did animals in which this anesthetic was injected directly into the peritoneal cavity. The results demonstrate that sodium amytal is absorbed both from the abdominal cavity and from the amniotic sac of the fetuses with relative rapidity. Furthermore, approximately the entire amount

passes through the placenta. This conclusion was further substantiated by the fact that when the fetuses were injected with an amount calculated as a minimal lethal dose for the mother, the latter died after a few minutes in 75% of the cases.

We conclude: 1. The amount of sodium amytal necessary to anesthetize a pregnant rat does not in any way interfere with the viability of the fetus; the fetus is not anesthetized, at least not completely, and readily responds to gross stimulation. 2. Sodium amytal quickly passes from fetal circulation into the maternal circulation. 3. In calculating the amount of sodium amytal necessary to anesthetize a pregnant animal, the weight of the fetuses must be subtracted from the weight of the mother.

5384

A New Capillary Hematocrit.

PAUL D. ROSAHN. (Introduced by L. Pearce.)

From the Laboratories of the Rockefeller Institute for Medical Research.

The apparatus to be described is an accurate and inexpensive hematocrit of extremely simple construction, employing heparin as an anticoagulant. Values are expressed directly in per cent by measuring the column of packed blood cells with a millimeter scale. That readings are made directly is an obvious advantage over a somewhat similar hematocrit described by Epstein,[1] with which the readings are calculated by dividing the length of the column of cells by the length of the original column of blood, and multiplying the quotient by 100.

The hematocrit here described is a pipette 12 cm. long, cut from selected glass tubing of uniform bore, having an outside diameter of 5 mm. and a bore of 0.5 to 0.8 mm. One end is beveled, and exactly 10 cm. from this end is engraved a circular mark. Before use the tube is lined with the anticoagulant by drawing an aqueous solution of ½ to 1% heparin through the pipette which is then allowed to dry. The tube is then sealed by encircling it with a rubber band No. 84 which is 9 cm. long and 1.3 cm. wide. No subsequent leakage occurs if this sized rubber band is placed securely around the pipette.

A rubber mouth suction tube is attached to the unbeveled end of

[1] Epstein, A. A., *J. Lab. and Clin. Med.*, 1915, **1**, 610.

the hematocrit, and freshly flowing blood obtained by needle prick is drawn up to the circular mark. Blood adhering to the outside is wiped away, the suction tube is carefully removed, and the pipette encircled from end to end with the rubber band. The hematocrit is now placed in a centrifuge with beveled end down and rotated for 20 minutes at 3000 r.p.m. Centrifugalization at this rate for more than 20 minutes gives constant readings. The red cells are completely sedimented, and clearly and sharply defined from the supernatant plasma. The column of red cells is then measured against a millimeter scale. Since the original length of the column of blood was 100 mm., the length of the packed red cells in millimeters gives directly its percentage. Similarly, the amount of plasma is expressed in per cent. After use, the tube is cleaned in the usual manner with water, alcohol, and ether, and when relined with heparin, is again ready for use.

Rabbit's blood has been employed in the standardization of this hematocrit. A series of consecutive readings on 10 animals, using 10 pipettes for each animal, has given a standard deviation ranging from a minimum of 0.2% to a maximum of 0.8%, with an average for the 100 readings of 0.5%. In terms of coefficient of variation, the range was from a minimum of 0.6% to a maximum of 2.2%, with an average for the 100 readings of 1.2%.

The accuracy of this method, as indicated by the small technical error of 1.2%, together with the simplicity and inexpensiveness of the pipette, and the ease with which readings are made directly in per cent, are features which recommend the hematocrit for use in the clinic and laboratory.

5385

Absorption and Elution of Antibodies from Various Antisera.

LEO OLITZKI AND MAX FRANKEL. (Introduced by I. J. Kligler.)

From the Departments of Hygiene and Biochemistry, Hebrew University, Jerusalem.

Frankel and Olitzki[1] showed that by specific absorption and elution it is possible to obtain antibodies free from protein by absorbing the antisera with kaoline, centrifugalizing and then eluting the kaoline absorbate with appropriate eluates. The eluates thus ob-

[1] Frankel, M., and Olitzki, L., *Nature*, 1930, **126**, 723.

tained are free from protein, in so far as they can be demonstrated by sensitive chemical tests, but contain active antibodies.

We here report experiments dealing with the elution of antitoxin, agglutinins, complement-fixing and lytic amboceptors and the behavior of these eluted antibodies. Since it is generally accepted that biological sensitization is a more sensitive test for proteins than the most sensitive chemical test, we attempted to ascertain by this method whether the eluates contained demonstrable proteins. The eluting fluid consisted of a 2% solution of glycocol with varying concentrations of sodium chloride. The technique was as follows:

Equal parts of antiserum (diluted 1:10) and kaoline were thoroughly shaken, allowed to stand 24 hours at 37°C. and centrifugalized. The kaolin-protein sediment was then resuspended in the eluting fluid and the suspension kept at 37°C. for 48 hours, then centrifugalized and the supernatant fluid tested for antibodies. The antitoxin content of the protein-free eluates was determined by the intracutaneous neutralization test employed by Roemer[2]; the content of agglutinin, complement-fixing and lytic amboceptors was measured by the methods described by Olitzki.[3]

The results showed that the largest amounts of antitoxin and of the flagellar typhoid agglutinin are obtainable in eluates made with 2% glycocol in 1 to 2% sodium chloride solution, while the largest amounts of the somatic agglutinins as well as the lytic and complement-fixing amboceptors were obtained in eluates made with 0.2 to 0.8% solution of sodium chloride. Attempts to elute by this method the complement-fixing amboceptors from luetic sera giving a strong positive Wasserman and Kahn reaction yielded negative results.

Since antibodies are generally considered to be bound up with globulins, it appeared of some importance to ascertain whether the antibody containing eluates were from the biological viewpoint protein-free as shown by the less sensitive chemical tests. We attempted to test this by sensitization of guinea pigs and subsequent anaphylactic test.

Six guinea pigs received subcutaneous injections of 1 cc. agglutinin-eluate (titre 1000 for *B. typhosus,* H-type) obtained in the manner described above. Sixteen days afterwards 3 of these guinea pigs received an intravenous injection of 0.5 cc. of this eluate, the other 3 an injection of the corresponding amount of the original serum (0.5 cc. serum diluted 1:10). There was a drop in body

[2] Roemer, F., *Z. für Immunitätsforschung und Exp. Therap.,* 1909, **3**, 308.

[3] Olitzki, L., *Centralblatt für Bakteriol.,* 1928, **106**, 247.

temperature in all the animals lasting 15-20 minutes, after which it returned to normal. Three control animals sensitized on the same day with 1.0 cc. of the original serum, diluted 1:10, showed after the intravenous injection of 0.5 cc. of the same serum dilution a heavy anaphylactic shock, associated with a decrease of the body temperature to below 35°C. which returned to normal only after 45 minutes.

In a second set of experiments the sensitizing injections consisted of 3 cc. of the same eluate; a control group received 3 cc. of the serum diluted 1:10. The second intravenous injections were carried out with 1.0 cc. of the eluate and with 0.5 cc. and 1.0 cc. respectively of undiluted serum. The animals treated with eluate showed a small drop in the body temperature lasting 15 minutes, while those treated with the original serum suffered a severe shock accompanied by a decrease of the body temperature to below 35°C. lasting 1 to 1½ hours. An intravenous injection of the eluate, or of a corresponding glycocol-sodium chloride solution, into untreated guinea pigs gave the same drop in body temperature as that noted in treated animals. It is apparent, therefore that the injection of glycocol eluates did not induce sensitization either against the injection of eluate or of original serum.

Repeated injections of eluates into rabbits (*i. v.*, 0.5 cc., 1.0 cc. and 1.5 cc.) did not yield precipitins either against the eluate or against the original serum.

It may be concluded that the eluates of antibodies found by sensitive chemical tests to be free from proteins have neither sensitizing nor precipitinogenic properties.

5386

Antigenic Power of Ultra-Violet Irradiated Tetanus Toxin.

EMERSON MEGRAIL AND HENRY WELCH.

From the Department of Hygiene and Bacteriology, Medical School, Western Reserve University.

It has been shown by one of us[1] that tetanus toxin is destroyed by a few minutes' exposure to ultra-violet light if toxic broth be diluted to decrease the concentration of protein and reduce the color absorptive factor. Previously, however, it has not been deter-

[1] Welch, H., *J. Prev. Med.*, 1930, **4**, 295.

mined whether toxin so treated retains its antigenic potency. Lowenstein's[2] tetanus toxin, treated with 0.2% formalin and subjected to ultra-violet light was atoxic and antigenic, but since it is now well known that formalin alone renders certain toxins atoxic while preserving their antigenic powers, the effect of the irradiation in his experiments is uncertain. An experiment was therefore undertaken using as an immunizing agent tetanus toxin rendered atoxic by ultra-violet light. A mixture of 2 tetanus toxins after titration was diluted to contain 1 M.L.D. in each 1 cc. This was irradiated with "C" carbons (National Carbon Co.) at a distance of 25 cm. in the apparatus described in a previous publication,[3] keeping the toxin below room temperature by a current of cool air.

A 2-minute irradiation period failed to destroy the toxin completely, as about half of the inoculated animals developed late tetanus. A further irradiation of 2 minutes rendered the material atoxic. Using not more than a quantity originally containing 1 M.L.D, 9 guinea pigs were given 5 subcutaneous injections of this irradiated toxin at 6 or 7 day intervals and were inoculated 3 weeks later with freshly titrated tetanus toxin in doses of from 1 to 10 M.L.D.'s. No signs of tetanus developed. The period of observation was 45 days.

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Hematopoietic Function in Avitaminosis. IV. Further Studies of Vitamin A Deficiency.*

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From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.

In a previous publication[1] we have suggested the occurrence of a secondary anemia in vitamin A deficiency. Our results, however, were not conclusive. We, therefore, continued this investigation with 30 more animals, 18 of which received our vitamin A deficient ration 1749.† Twelve of these rats were allowed a modification of

[2] Lowenstein, E., *Z. Exp. Path. u. Ther.*, 1914, **15**, 279.

[3] Perkins, R. G., and Welch, H., *J. Prev. Med.*, 1929, **3**, 363.

* Research Paper No. 198, Journal Series, University of Arkansas.

[1] Sure, B., Kik, M. C., and Walker, D. J., *J. Biol. Chem.*, 1929, **83**, 375.

† Composition of ration 1749 is as follows: Casein (hot-alcohol extracted), 20; N. W. yeast, 10; McCollum's salts No. 185 (2), 4; lard, 2; dextrin, 64. This ration was irradiated for 30 minutes with a mercury quartz vapor lamp to insure an adequacy of vitamin D.

this diet, so that one to 2% of lard was replaced by equivalent amounts of butterfat, in order to prevent sudden deaths from pneumonia frequently associated with this avitaminosis. In addition, we have studied the effect of vitamin A deficiency on the differential leucocyte count in 9 females and 9 males. In the females the disease was followed with daily examinations of the vaginal smears according to the technique of Evans and Bishop.[3] Daily records were kept of food consumption, and in the majority of animals records were also kept of the daily water intake. Bleedings were performed on each animal twice weekly and determinations were made of hemoglobin and erythrocyte counts. Specific gravity determinations were also made, in order to secure information on blood concentration. The experimental period ranged from 68 to 173 days.

To summarize our results, we found no noteworthy disturbance in hematopoietic function during various stages of this avitaminosis, as characterized by the severity of ophthalmia and loss of body weight, nor any changes in the differential leucocyte count during the onset, and remission produced by vitamin therapy. Our results, therefore, do not substantiate the findings of Koessler and coworkers.[4] Although secondary anemias have been encountered in keratomalacia in man,[5] the investigators admit that other complications or infections associated with this disease may be the influencing factors rather than this avitaminosis.

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Hematopoietic Function in Avitaminosis. V. Vitamin D Deficiency.*

BARNETT SURE AND M. C. KIK.

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In a study of the secondary anemia of childhood, Happ and Evans[1] pointed out that 9 of their 10 patients had rickets, although

[2] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, **33**, 63.

[3] Evans, H. M., and Bishop, K. S., *J. Metabol. Res.*, 1922, **1**, 10.

[4] Koessler, K. K., Maurer, S., and Loughlin, R., *J. Am. Med. Assn.*, 1926, **87**, 476. Koessler, K. K., and Maurer, S., *J. Am. Med. Assn.*, 1927, **89**, 768.

[5] Keefer, C. S., and Yang, C. S., *Nat. Med. J., China*, 1929, **15**, 419.

* Research Paper No. 199, Journal Series, University of Arkansas.

[1] Happ, W. M., and Evans, F. A., *Johns Hopkins Hosp. Bull.*, 1922, **33**, 7.

the anemia could not be attributed to a lack of vitamin D, since it did not improve following the addition of cod liver oil. Since in human cases we seldom encounter uncomplicated avitaminosis, for the reason that diets that are deficient in one or 2 vitamins are generally deficient in other respects, particularly in minerals, we have found it of interest to determine if an anemia could be produced on the Steenbock-Black ration No. 2965[2] which is now extensively used by nutritional workers in vitamin research for the production of uncomplicated rickets in the rat.

For this study we have employed 39 animals, 30 on the Steenbock and Black ricketic diet No. 2965 and 9 controls receiving the same diet supplemented with vitamin D by irradiating the ration 30 minutes with a mercury quartz vapor lamp.

TABLE I.

Representative Case of Hematopoietic Function on Steenbock and Black Ration No. 2965, Irradiated for 30 Minutes with a Quartz Mercury Vapor Lamp (♀ 6579).

Date (1929)	Age (days)	Weight (gm.)	Hemoglobin Erythrocytes.		Specific Gravity
			Index Gm. per 100 cc.	Count R. B. C. per mm.^{3}	
Jan. 29	36	52	11.2	6.1	1.0545
Feb. 5	43	58	9.6	6.2	1.0470
12	50	65	9.5	7.1	1.0493
19	57	71	7.7	6.6	1.0458
27	65	75	8.2	6.7	1.0448
Mar. 5	71	77	7.8	7.0	1.0461
12	78	88	8.4	7.3	1.0395
19	85	93	9.2	7.2	1.0427
26	92	96	8.0	6.2	1.0387

Bleedings of most of the rats were performed twice weekly and of the others once weekly. Daily records were kept of body weight and food intake. At each bleeding hemoglobin and specific gravity determinations and erythrocyte counts were made. In Table I we present the hematopoietic function of a representative animal receiving the Steenbock and Black ration 2965 supplemented with vitamin D, showing anemia, as evidenced by the concentration of hemoglobin and erythrocyte counts. This is the typical picture that was obtained on the ricketic animals as judged by the line tests,[3] and the

[2] Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

[3] Shipley, P. G., Park, E. A., McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1921, **45**, 343.

same blood picture was encountered on all the 9 control animals that showed normal calcification. We, therefore conclude that vitamin D deficiency has no influence on hematopoietic function.

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Hematopoietic Function in Avitaminosis. VI. Vitamin G Deficiency.*

BARNETT SURE, M. C. KIK AND MARGARET ELIZABETH SMITH.

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That pellagra is an avitaminosis, produced by a deficiency of a dietary factor, associated with the vitamin B complex, is now generally recognized. Recently, however, Bliss[1] has advanced the theory that pellagra is an iron deficiency disease and claims that the curative agent of the vitamin-containing foods employed by Goldberger and other investigators is not the "so-called vitamin G" but iron. Although it has not yet been conclusively established whether the pellagra-like disease in the rat is the analogue of human pellagra or not, we trust that the character of the results of our studies on the hematopoietic function in vitamin G deficiency in the rat may be of interest to investigators in this field.

The dietary regime for the production of vitamin G deficiency has been described elsewhere.[2, 3] The optimum ration for the production of dermatitis was found to be one deficient in the vitamin B complex, supplemented by a daily allowance of 500 mg. of rice polishings, and irradiated for 10 hours, in order to insure the destruction of the greater portion of vitamin G.[4] We have found no specific relation between failure in growth and the incidence of pellagra-like symptoms in the rat, the dermatitis being prevalent in some animals that made normal growth and absent in others that entirely failed in growth and finally collapsed following great losses of weight. The latter phenomenon we encountered most frequently when our basal diet was supplemented with alcoholic extracts from whole wheat, as a source of vitamin B. We, therefore, suggested

* Research Paper No. 200, Journal Series, University of Arkansas.

1 Bliss, S., *Science*, 1930, **72**, 578.

2 Thatcher, H. S., Sure, B., and Walker, D. J., *So. Med. J.*, 1930, **23**, 143.

3 Thatcher, H. S., and Sure, B., *Arch. Path.*, 1931, in press.

4 Hogan, A. G., and Hunter, J. E., *J. Biol. Chem.*, 1928, **78**, 433.

that the antidermatitis factor is a syndrome distinct from the growth-promoting substance, both of which are associated with "so-called vitamin G."[5]

A total of 44 animals was employed for this study. Bleedings were performed once to twice weekly and determinations were made of hemoglobin and specific gravity, and erythrocyte counts. Records were also kept of food and water intake. The experimental period lasted from 76 to 147 days.

The results on animals that showed loss of weight, unaccompanied by dermatitis, are as follows: Twenty-two animals were used in this investigation, 16 pathological, and 6 positive controls. We found no noteworthy changes in the concentration of hemoglobin or erythrocytes in 10 animals. The anemias observed in 3 animals may be attributable to inanition, since anorexia became quite pronounced in these rats. Three animals, however, which showed a normal food intake and which were apparently in excellent state of nutrition, showed a reduction of 25 to 50% in the concentration of hemoglobin.

Our results on 14 animals, showing dermatitis, compared with 8 positive controls, may be summarized as follows: Ten out of 14 animals showed marked anemia. In 2 cases the reduction of erythrocytes ran parallel to the decrease of hemoglobin, both of which occurred in animals that developed marked anorexia. In the rest of the cases the anemia was due mainly to the reduction of hemoglobin to the extent of 30 to 50%. The latter animals were consuming 7 to 8 gm. of the ration daily, which is considered normal for the rat.

We found no significant changes in the total and differential leucocyte count in this avitaminosis.†

While our results are somewhat irregular, it would seem that an anemia occurs in vitamin G deficiency when accompanied by skin lesions comparable to that found in human pellagra. Since our diets had an abundance of ferric citrate, and since in such anemia we found no response to administration of the ash from yeast, we conclude that the anemia we encountered is not produced by a mineral deficiency.

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[5] Sure, B., Smith, M. E., and Kik, M. C., *Science*, 1931, in press.

† Credit is due Miss Dorothy J. Walker for making the total and differential leucocyte counts.

5390

Effect of Estrin Injections on the Growth Curve of Young Rats.

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From the Research Laboratories of the University of Denver.

Moore and Price[1] injected male and female sex hormones into normal and castrate rats. They concluded that gonadal hormones suppress the functions of the hypophysis in the production of gonad stimulating secretions. Working with the female sex hormone, we wished to learn whether or not this inhibition of the hypophysis extended also to the growth-promoting factor produced by this gland. We therefore injected a very pure and carefully assayed preparation into young rats, and studied their growth curves.

The hormone preparation was obtained from pregnancy urine according to the method previously described,[2] except that no effort was made to obtain crystalline material. It was assayed according to the Coward and Burn method, as repeated by us,[2] and the rat unit found to be 5 gamma. Three litters of young rats, 24 in all, were divided so that equal numbers from each litter would be used in the control and experimental groups. Each group included 12 animals, 6 males and 6 females. The experiment was started immediately after the animals were weaned. The experimental group received 40 units of hormone dissolved in 0.2 cc. of olive oil every other day, the control group the same amount of olive oil only. The animals were weighed every week and their growth plotted.

Results: A small but, we believe, significant difference in the growth rates of the 2 groups was observed. The growth rates were consistent, in no case did any injected animal grow as rapidly as any control animal. The injected group gained 1051 gm. or 211.0%. The non-injected group gained 1348 gm. or 262.3%.

It was observed that the testes of the injected animals appeared much smaller than those of the control group, therefore all animals were autopsied and the sex glands weighed. The results are striking and again uniformly consistent, in every case the ratio of testes (or ovary) weight to body weight is very much smaller in the injected group. The average weight of the ovaries of injected animals is 43% of the normals and the average weight of testes of injected animals only 29% of the normal.

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[1] Moore, C. R., and Price, D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 38.

[2] D'Amour, F. E., and Gustavson, R. G., *J. Pharm. and Exp. Therap.*, 1930, **40**, 473.

Examination of the ovaries revealed mature follicles and corpora lutea in the case of the normal animals while those of the injected animals were pale and showed no signs of maturity. No histological studies were made.

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Use of Organic Acids for the Differentiation of Salmonella pullorum and Salmonella gallinarum.

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Brown, Duncan and Henry[1] demonstrated that the sodium salts of 4 organic acids, tartaric, mucic, fumaric and citric, were utilized as food by some members of the paratyphoid group, thus making it possible to separate certain members of this group through the use of this agency. The utilization of the acids as foods was measured in a liquid medium by precipitation of the residual acid by lead acetate. Using this procedure they were able to separate *Salmonella aertrycke* and *Sal. schotmülleri*. In a personal communication, Henry and Duncan[2] reported the separation of *Sal. pullorum* and *Sal. gallinarum* as follows:

Organism	1% dextrotartrate	0.5% laevotartrate
Sal. pullorum	—	+
Sal. gallinarum	+	—

Jordan and Harmon[3] demonstrated that sodium tartrate peptone medium containing phenol red separated *Sal. aertrycke* and *Sal. schotmülleri* by a color change due to difference in pH.

Using a method of procedure similar to that of Jordan and Harmon, the writer tested the sodium salts of citric, d-tartaric, fumaric and mucic acids on *Sal. pullorum* and *Sal. gallinarum* together with a few closely related organisms. Fumaric and citric acids were found unsatisfactory as the pH changes induced were very inconstant. On the other hand, the reactions obtained with d-tartaric and mucic acids were very constant. The data on the latter 2 acids are presented in Table I.

[1] Brown, H. C., Duncan, J. T., and Henry, T. A., *J. Hyg.*, 1924, **23**, 1.

[2] Personal communication from Dr. H. C. Brown in 1927.

[3] Jordan, E. O., and Harmon, P. H., *J. Infect. Dis.*, 1928, **42**, 258.

TABLE I.
The fermentation of d-tartaric and mucic acids by members of the Salmonella group as measured by pH changes.

Organism	Sodium d-tartarate		Sodium mucate	
	alkali	acid	alkali	acid
Sal. pullorum	+	—	+	—
Sal. gallinarum	—	+	—	+
Shig. jeffersonii	—	+	—	+
Sal. schotmülleri	+	—	—	+
Sal. aertrycke	—	+	—	+
Eberth. typhi	+	—	+	—

The data show that both sodium salts of d-tartaric and mucic acids differentiated between *Sal. pullorum* and *Sal. gallinarum.* Using these salts on a number of strains of both organisms over a period of 3 years, no variations or exceptions in the reactions listed above were obtained with either the tartrate or mucate media. Liquid and agar stab cultures gave the same reactions. The data on *Sal. aertrycke* and *Sal. schotmülleri* confirmed the work of Jordan and Harmon. The identical reactions obtained with *Sal. gallinarum* and *Shig. jeffersonii* add strength to the statement of St. Johns-Brooks and Rhodes[4] that these 2 organisms are identical.

5392

Evidence of Biologic Relationships Among Species of Chenopodiales.

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The differentiation of plants into species or larger groups is usually based upon gross or microscopic characteristics. Physiologic criteria are seldom emphasized and chemical ones are notoriously inadequate in the establishment of significant differences. The order *Chenopodiales* contains several genera which have been implicated in clinical allergy in man. The more important of these are *Amaranthus, Atriplex, Chenopodium* and *Salsola.* All, except the first, are included in the family *Chenopodiaceae.* There are many anatomic characteristics common to these genera although the gross appearances in the individual species differ enormously.

[4] St. Johns-Brooks, R., and Rhodes, M., *J. Bacteriol. and Path.*, 1923, **26**, 433.

Approximately 300 allergic patients (62 in the Santa Fe group), drawn from the area commonly designated as the Pacific Southwest, have been tested with the same extract of pollen from one or more species of each genus listed above. A significant number of these patients, especially those from the desert sections, gave skin reactions to these extracts. The total number of Chenopodiales-sensitive patients in each of the 3 groups is essentially the same. Those from the Santa Fe Clinic, however, represent 74% of the total patients in that series as compared with 57% and 38%, respectively in the 2 remaining groups. The higher incidence of sensitivity to this type of flora among Santa Fe patients may be due to greater exposure to these pollens.

The object of this study is not to draw sweeping conclusions but rather to point out interesting possibilities, based on skin reactions, in the hope that others may confirm or nullify these suggestions. *Amaranthus retroflexus* seems to be rather closely related to all the species studied. The fact that it reacted alone on but 4 patients of the 144 sensitive to *Chenopodiales*—in a total of 278 patients—seems to indicate a high degree of specificity of the extract. If reactions are obtained to it one should suspect sensitivities to other members of this order, although the absence of a reaction does not exclude other sensitivities. In the Santa Fe group but 22% of the patients, as compared to 51% and 47% in the other groups, failed to react to this pollen and did react to other members of the order. On the other hand 80% of the Santa Fe patients, as compared to 43% and 51% of the other groups, reacted to *A. retroflexus* and to one or more additional members; in fact, from 50% to 80% of these individuals reacted to all other genera.

The subdivisions of these totals, in most instances represent but few observations, yet they suggest that *Salsola kali* has little "anti-

TABLE I.—Skin reactions to species of the botanic order *Chenopodiales*.

	Total pts. sensitive to *Chenopodiales*	Reactions to *Amaranthus retroflexus* only	Patients *not* reacting to *Amaranthus retroflexus* but to the following—								Patients reacting to *Amaranthus retroflexus* and to the following—							
			Atriplex only	Chenopodium only	Salsola only	Atriplex and Chenopodium	Atriplex and Salsola	Chenopodium and Salsola	Atriplex, Chenop. and Salsola	Total patients	Atriplex only	Chenopodium only	Salsola only	Atriplex and Chenopodium	Atriplex and Salsola	Chenopodium and Salsola	Atriplex, Chenop. and Salsola	Total patients
Sante Fe	46	0	0	0	2	2	0	0	6	10	0	1	0	2	2	0	31	36
General Hospital	47	3	6	5	1	3	0	3	6	24	1	3	1	1	1	3	10	20
Private	51	1	2	5	4	7	1	2	3	24	2	1	0	6	3	1	13	26

genic" individuality. Sensitivity to it alone, or in combination with but one other genus seldom occurs. In the total of 58 patients who did not react to *A. retroflexus* 57% reacted to *S. kali.* In the group reacting to *A. retroflexus* 79% of the total reacted also to *S. kali.* It thus appears that the pollen of *S. kali* and *A. retroflexus* possesses one or more factors—possibly similar to group antigens among bacteria—common to the majority of the other species. In view of the fact that but 25 patients (less than 20%) of the total of 144, reacted to but one of the 3 genera—*Atriplex, Chenopodium* and *Salsola*—it is suggested that the pollen extract of each species contains many minor "antigenic" properties typical of the great majority of the other species, even though they be members of different genera. These findings apparently lend support to the botanic classifications and indicate a method for studying biologic relationships.

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Persistence of Accelerated Rate in Isolated Hearts of Thyrotoxic Rabbits: Response to Iodides, Thyroxine and Epinephrine.

J. K. LEWIS* AND DONALD MC EACHERN.† (Introduced by Edw. P. Carter.)

From the Cardiographic Laboratory of the Johns Hopkins Hospital and University.

Acute experiments with thyroid products, either on the intact animal or upon isolated tissues, have been uniformly unsuccessful in reproducing the phenomena of hyperthyroidism. The advent of crystalline thyroxine has demonstrated that most of the non-specific results have been due to proteins and other impurities in the material used. In hyperthyroidism induced by thyroid feeding and in spontaneous hyperthyroidism in the human, tachycardia is one of the most characteristic features. It has been ascribed variously to mechanical causes, to nervous influences, to the overwork incident to a heightened metabolism, to toxic damage of the heart and to an increased production of epinephrine. Upon removal of the cause of hyperthyroidism or discontinuance of thyroid feeding the heart rate falls gradually, over a number of days, to normal. During the course of the experiments reported below it was found that the hearts and auricles of thyroid-treated animals, when isolated, continued to beat at a much faster rate than similar preparations ob-

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† Jacques Loeb Fellow in Medicine.

tained from normal animals. This seemed to indicate the persistence of the specific thyroid effect on the isolated tissue and was the basis for a comparison of certain pharmacological reactions of the two types of hearts.

Healthy rabbits were used in all experiments. Some were fed thyroid gland (desiccated and powdered) in doses varying from 0.104 to 0.390 gm. daily over periods varying from 2 to 81 days. Others received Thyroxin (Roche) by intramuscular injection every second day in doses sufficient to average from 0.05 to 0.15 mg. daily over periods varying from 6 to 25 days. Weight, heart rates and temperatures were followed at frequent intervals. The usual tachycardia, rapid loss of weight, tachypnoea and sometimes increase of temperature and diarrhoea were observed. The animals were killed by a blow on the head and the hearts quickly removed and set up in the Lehendorff apparatus and perfused with oxygenated Ringer-Locke's solution at 35°C. and adjusted to pH 7.4. In other experiments the auricles were dissected free under iced Ringer-Locke's solution and set up in a Dale bath of 100 cc. capacity which could be quickly emptied and refilled with Ringer-Locke's solution adjusted to pH 7.4 and kept at 35°C. Warmed oxygen was bubbled continuously through the solution in which the auricles were suspended.

The contractions of the preparations were recorded on a revolving smoked drum. Spontaneous rhythm commenced in every instance upon perfusion with or immersion in the warm Ringer-Locke's solution and persisted until the experiment was terminated. Control tracings were first made and the conditions of the experiment then altered by the addition of different drugs and solutions. Determinations of the pH of the solutions were made frequently. Coronary flow was measured directly by collecting the perfusate over known periods of time as it was discharged from the heart.

Results. Averages of the maximum rates of the normal preparations and those from thyroid treated animals will be found in Table I. They were registered early in each experiment after the preparations had settled down. The usual tendency to a gradual

TABLE I.

Average Maximum Rate per Minute of the Isolated Hearts and Isolated Auricles of Normal, Thyroid-fed and Thyroxinized Animals.

	Normal	Thyroid-fed	% Inc.	Thyroxinized	% Inc.
Isolated Hearts	145 (16 Exp.)	224 (6 Exp.)	54.4	183 (6 Exp.)	26.2
Isolated Auricles	125 (21 ")	187 (5 ")	49.6	—	

decrease in rate over a period of hours was observed in each instance, the decrease being of similar degree both in normal preparations and those from thyroid-treated animals.

It is obvious that the preparations from hyperthyroid animals beat at a much faster rate than those from normal animals. This was a constant and striking phenomenon which persisted throughout each experiment.

The effects of various drugs and solutions are summarized below: Iodine and iodides in concentrations of 1:100,000 to 1: 4,000,000 had no significant effect on the isolated auricles but caused moderate decrease in rate of 5 of 11 and in amplitude of 8 of 11 isolated hearts. There was no difference in the response of normal preparations and those from thyroid-treated animals. No constant changes were observed in coronary flow.

Thyroxine and desiodothyroxine, in acute experiments, had no constant effect on normal preparations or on those from thyroid-treated animals.

Preparations from thyroid-treated animals repsonded to epinephrine with increases in rate and amplitude *(percent)* similar to those of the normals. Acceleration of rate *(beats per minute)* was greater in the former preparations than in the normals. There was no difference in sensitivity to high dilutions (1:100,000,000) of epinephrine.

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Effect of Withdrawal of Vitamin-A on Leukocyte and Differential Count in the Albino Rat.

R. G. TURNER AND E. R. LOEW. (Introduced by E. W. Rockwood.)

From the Department of Medical Research, Detroit College of Medicine and Surgery.

It is generally accepted that animals deprived of Vitamin A develop a characteristic susceptibility toward infection.[1] Suppurative lesions of the upper respiratory tract, sinuses and middle ear occur

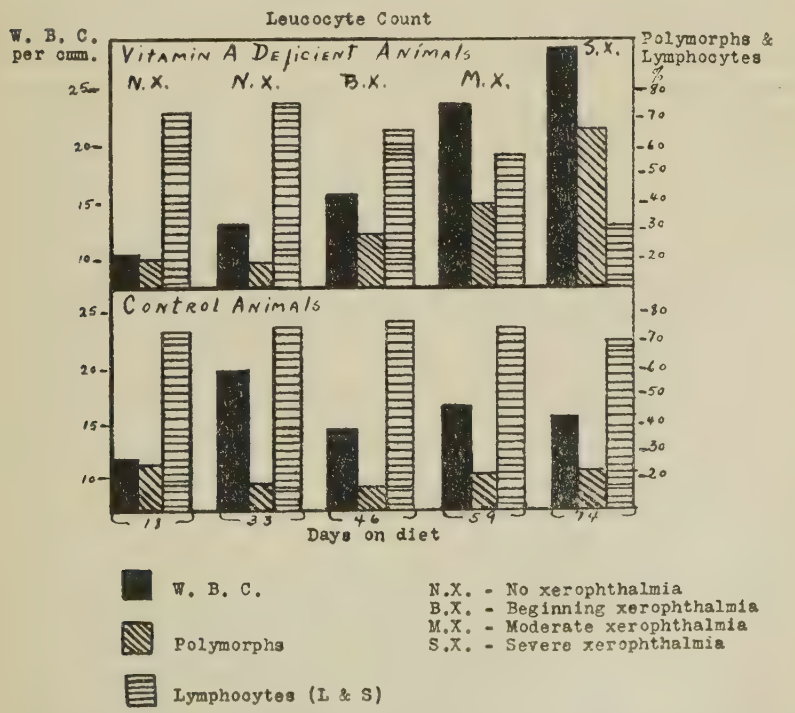
[1] McCollum, E. V., *J. Am. Med. Assn.*, 1917, **68**, 1379; Drummond, J. C., *Biochem. J.*, 1919, **13**, 95; Macy, Icie G., Outhouse, J., Long, M. L., and Graham, A., *J. Biol. Chem.*, 1927, **78**, 152; Sherman, H. C., and Burtis, M. P., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 649.

rather frequently in rats deprived of this nutritional element.[2] The relation of this characteristic as compared to the leukocyte and differential count at various stages of the disease is of interest.

Cramer, Drew and Mottram[3] observed a lympho-paenia in the circulating blood of rats placed on diets deficient in water-soluble vitamin B. They found no change in the differential or W. B. C. count in rats placed on vitamin A deficient diet. Their report includes data on only 2 rats fed on diets lacking vitamin A, at 50 to 70 days of age, for a period of 6 to 7 weeks. One of the animals developed xerophthalmia, the other did not. From the growth curves presented for these animals they could not have shown more than a mild form of the disease as they were still gaining weight at the end of the experiment.

Our animals were studied until they showed severe symptoms of vitamin A deficiency with marked loss in weight and gross xeroph-

Chart No. 1.



[2] Daniels, Amy L., *J. Am. Med. Assn.*, 1923, **81**, 828; Turner, R. G., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 233; Shurly, B. R., and Turner, R. G., *J. Am. Med. Assn.*, 1930, **94**, 539; Turner, R. G., Anderson, Dorothy E., and Loew, E. R., *J. Infect. Dis.*, 1930, **46**, 328.

[3] Cramer, W., Drew, A. H., and Mottram, J. C., *Lancet*, 1921, **2**, 1202.

thalmia. Total leukocyte count and differential count of the blood of rats during various stages of the disease are reported for 13 xerophthalmic animals and 5 control animals. A graphical diagram is given in Chart 1.

The average white blood cell count for animals placed on vitamin A deficient diet did not show any increase over the average count of the controls on the 46th experimental day. At this time most of the animals showed a mild form of xerophthalmia. At 59 days the average count shows an increase of 4,000 W. B. C. per cmm. over the highest average found in the controls. The animals at this time showed a moderate xerophthalmia, both eyes affected, with loss of weight. The average count for 6 animals remaining on the deficient diet for 74 days continued to show this increase in the W. B. C. These animals were very weak and emaciated and would probably have succumbed within several days from the effect of lack of vitamin A in their diet.

The average differential count taken on the 46th experimental day showed a slight increase in the relative percentage of polymorphs with a corresponding decrease in the lymphocytes for the vitamin deficient animals. This change became more marked as the animals remained on the diet. In the animals showing gross pathological symptoms the increase in polymorphs reached 67% while the lymphocytes dropped to 31%. The relative percent of polymorphs and lymphocytes as found in the control animals remained nearly constant throughout the experimental period. These findings show the normal lymphocyte count to constitute about 75% of the total white blood cells in the rat, the polymorphs averaging about 20%. Cramer, Drew and Mottram[3] found the percentage value of small lymphocytes to be about twice that for the polymorphonuclear leucocytes in the rat. The lymphocyte count in the data presented includes both large and small lymphocytes. The stain used for differentiating was Wright's stain. The Wistar Institute[4] reports contrary findings for the normal rat, *i. e.,* a higher percentage of polymorphs than lymphocytes.

Variations in the leukocyte count of control animals may be interpreted as possible fluctuating differences due to physical and metabolic activities. Müller, Peterson and Holscher[5] believe that many factors other than disease may produce significant physiologi-

[4] The Rat: Memoirs of the Wistar Institute of Anatomy and Biology No. 6. H. H. Donaldson, 2nd Edition, 1924.

[5] Müller, E. F., Peterson, W. F., and Holscher, Rose, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 544.

cal fluctuations of considerable magnitude. Garrey and Butler[6] believe that passive or active postural changes raise the count instantly from 60 to 100% in humans. The normal leukocyte count for albino rats on a complete vitamin A synthetic diet (controls) may range from 9,000 to 24,000 W. B. C. per cmm., the highest average being 20,000, The highest counts obtained on the 33rd experimental day, did not remain constant and at no other time in the experiment reached as high a limit. The final counts taken on all animals are summarized as follows:

20%	of	the	control	animals	showed	a	count	between	9,000	and	10,000	per cmm.
80%	"	"	"	"	"	"	"	"	10,000	"	20,000	" "
7%	of	the	diseased	"	"	"	"	"	9,000	"	10,000	" "
38%	"	"	"	"	"	"	"	"	10,000	"	20,000	" "
30%	"	"	"	"	"	"	"	"	20,000	"	30,000	" "
25%	"	"	"	"	"	"	"	"	30,000	"	50,000	" "

Fifty-five percent of the animals placed on vitamin A deficient diet showed a leukocytosis as compared with the W. B. C. findings in the control animals. The xerophthalmic animals likewise showed a reverse picture in the relative percent of polymorphs and lymphocytes as compared to the controls, and as found in the test animals before the onset of xerophthalmia, an increase in the polymorphs with a decrease in the lymphocytes, becoming more noticeable as the disease becomes more severe. Control animals showed a higher percentage of lymphocytes than polymorphs throughout the experiment. The generally accepted theory is that suppurations which develop in the cavities of these animals deprived of vitamin A are secondary to the metaplasia of cells and xerophthalmia. This theory apparently coincides with the variation found in the leucocyte counts. Metchnikoff's theory of phagocytosis suggests that the polymorphonuclear variety of cells is especially adopted to attack bacteria. Leukocytic defence of each individual rat studied begins after xerophthalmia sets in as noted by the increase in W. B. C. count. At the same time there is an increase in the multicellular leucocytes. This increase in polymorphs continues until the animal succumbs. The majority of bloods from xerophthalmic rats examined bacteriologically were found to be sterile. A very mild form of septicemia, undetected by bacterial culturing, may be the cause of increased phagocytosis. Various investigators[7] believe that upper respiratory, sinus and middle ear infections may be the direct cause

[6] Garrey, W. E., and Butler, Virginia, *Am. J. Physiol.*, 1929, **90**, 355.

[7] Smith, D. L., *South. Med. J.*, 1929, **22**, 918; Dixon, O. J., *J. Am. Med. Assn.*, 1928, **91**, 1280; Cone, A. J., *Laryngascope*, 1927, **37**, 19.

of numerous other systemic conditions brought about probably by a long continued mild form of septicemia. Evidence has accumulated that lymphocytes and monocytes play a rôle in promoting tissue repair. The experimental animals are all young growing rats and their need for tissue repair and rebuilding may be the cause of the high percentage of lymphocytes as found in the control animals.

The leucocytosis observed in the xerophthalmic animals together with an increase in the relative percent of polymorphonuclear cells and a decrease in the lymphocytes, logically, must be considered as a leucocytic defence of the individual animal to combat the invasion of virulent organisms. Is there then a lowered resistance of the specific membrane on which the organism gains control? Lowered resistance of the body is generally noted by an increase in the number of leucocytes, the phagocytes being less in number.

Conclusion. Leukocytosis with an increase in the percent of polymorphonuclear leukocytes and a decrease in the lymphocytes of the circulating blood is evident in the later stages of vitamin A deficiency in the rat.

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On the Cortical Hormone of the Adrenal Gland.*

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The crude lipid extract of beef adrenal cortex[1] and the aqueous extract[2] obtained from the active lipid fraction contain, with the cortical hormone, small quantities of adrenalin. The adrenalin content of the aqueous extract is sufficiently low to permit the demonstration of its efficacy in maintaining indefinitely the lives of adrenalectomized cats.[2, 3]

A simple method for the separation of adrenalin from the corti-

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Pfiffner, J. J., and Swingle, W. W., *Anat. Rec.*, 1929, **44**, 225; *Am. J. Physiol.*, 1931, **96**, 153.

[2] Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 321; *Am. J. Physiol.*, 1931, **96**, 164.

[3] Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 489.

cal hormone has been found.[4] The 70% alcohol soluble fraction obtained by our previously described method[2] is transferred to 95% alcohol and filtered through permutit. An extract with an adrenalin concentration of less than 1:2,000,000 (bio-assay-blood pressure) and a tissue equivalent of 30 gm. of cortex per cubic centimeter can be prepared by filtering through permutit twice using 20 gm. per kilo of tissue on the first filtration and 10 gm. per kilo on the second. The active material remaining in the permutit filter after each filtration is washed out with alcohol. The active fraction is transferred to water and the extract clarified by Seitz filtration. Besides adrenalin the permutit removes most of the contaminating pigment substances along with other inert material.

Intravenous injections of this type of extract have been used successfully in the crises of Addison's disease.[5]

Fractionation with permutit has made possible the preparation of active extracts from whole beef adrenal glands thereby doing away with the expense of dissection. These extracts have been found to be just as active in restoring prostrate adrenalectomized cats to apparently normal health as extracts prepared from dissected adrenal cortex. Whole adrenal gland extract (1 cc. equivalent to 50 gm. of whole gland) has an adrenalin content of approximately 1:2,500,000. This extract has been found suitable for subcutaneous, intraperitoneal and intravenous use. The solid content ranges in different batches from 0.3 to 0.4%.

Another method for separating adrenalin from the cortical hormone consists in distributing an active fraction between aqueous alkali and an immiscible solvent such as benzene or ether. Adrenalin passes into solution in the aqueous alkali whereas the cortical hormone is found in the immiscible solvent phase. By means of this fractionation step, highly active extracts (1 cc. equivalent to 30 gm. cortex) have been prepared containing less than one part of adrenalin in 4,000,000. This type of extract can be used subcutaneously, intraperitoneally or intravenously.

Aqueous cortical extracts are rendered inactive by boiling gently in an open flask for 2 minutes.

The unsaponifiable fraction obtained from the 70% alcohol soluble fraction referred to above, using sodium ethylate saponification, is completely inactive as tested on prostrate adrenalectomized cats.

[4] Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **72**, 75; *Am. J. Physiol.*, 1931, **96**, 180.

[5] Rowntree, L. G., Greene, C. H., Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **72**, 482; *J. Am. Med. Assn.*, 1931, **96**, 231.

The cortical hormone is removed from ether solution of an active fraction by washing with dilute acid.

Extract of beef adrenal cortex prepared with permutit fractionation gives a negative biuret, ninhydrin, Hopkins-Cole, Molisch, Pauly, and Liebermann Burchard reaction. It gives a positive xanthroproteic, Millon's, alkaline copper and alkaline phosphotungstate reaction. These 4 positive reactions can be accounted for by the presence of traces of phenolic decomposition products of adrenalin.

It has been found that aqueous extracts containing the cortical hormone can be preserved by the addition of benzoic acid in 0.1% concentration.

5396

A Precipitant for Material in Liver Active in Pernicious Anemia.

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The identification of γ hydroxyproline[1] among the products of hydrolysis of an acid active in pernicious anemia indicated the possibility of using Reinecke's[2] salt as a precipitant for this material from liver. Experiments have shown that Reinecke's salt does precipitate this acid and also precipitates active material from liver extract and promises to be useful as a preparative method. This observation is in harmony with the view that the γ hydroxyproline nucleus is an integral part of the active material in liver. The composition of the precipitate derived from the acid closely approximates that required for one molecule of organic acid and 2 molecules of Reinecke's acid.

[1] Dakin, H. D., West, R., and Howe, M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 2.

[2] Kapfhammer, J., and Eck, R., *Z. Physiol. Chem.*, 1927, **170**, 294.

5397

Study of the Virus of the Common Cold and Its Cultivation in Tissue Medium.*

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In previous papers[1] we reported the evidence which supports our belief that the common cold in man is caused by a filterable virus. This view is strengthened by recent work published by Long and Doull.[2] In addition to the effort to decide the question of etiology one of the objects of this investigation has been to explore the possibility of developing a specific prophylaxis for this disease. The first steps in the successful carrying out of such a procedure must naturally be directed toward an attempt to preserve the virus in an active form outside the animal body and an effort to cause this agent to reproduce itself *in vitro*.

In order to test survival of the virus after removal from the human body, naso-pharyngeal washings were obtained in the manner previously described, from individuals within the first 24 hours of a typical attack of common cold. The material was quickly passed through a Seitz filter and the filtrate was subsequently tested for its capacity to induce the symptoms of the common cold in chimpanzees and in human volunteers. The filtrates with cysteine hydrochloride added were preserved anaerobically under vaseline seal both at room and at ice-box temperature. In a few instances the filtrate was concentrated to approximately one-seventh its original volume by vacuum distillation. Of 12 inoculations of filtrates preserved in this manner 9 have produced in the inoculated subject typical symptoms of the common cold. The duration of time of preservation of active filtrates has ranged from 4 to 13 days. Tests of survival beyond the thirteenth day have not been made. Positive results have been obtained with filtrates kept at room temperature and with those kept at ice-box temperature, and with both the unconcentrated filtrate and with that concentrated by vacuum distillation. From these experiments we conclude that the virus of the common cold

* This investigation has been supported by a grant from the Chemical Foundation, Inc.

[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 562; **27**, 59; *J. Exp. Med.*, 1930, **52**, 701; Shibley, G. S., Mills, K. C., and Dochez, A. R., *J. Am. Med. Assn.*, 1930, **95**, 1553.

[2] Long, P. H., and Doull, J. A., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 53.

survives under suitable conditions for at least 13 days after removal from the human body.

In view of the capacity of this virus to survive in what might be considered an adverse environment an effort was next made to cultivate the agent in living tissue medium. Foster[3] has previously reported the cultivation of an organism from patients suffering from the common cold. The method of culture chosen by us has been that developed by Maitland and Maitland[4] and by Li and Rivers.[5] Ten day chick embryos are hashed and suspended either in Tyrode's solution or in buffered bouillon prepared from a special peptone† and to which 0.1% gelatin has been added. All solutions, both those used for naso-pharyngeal washing and for cultivation, contain 1 to 2000 cysteine hydrochloride. At all times an effort has been made to protect the agent against the deleterious action of peroxides. The cultures were incubated under vaseline seal at 37°C. for from 4 to 9 days. The material used for cultivation was obtained in the following manner: naso-pharyngeal washings were made from a patient with a typical cold of more than moderate severity. The washings were passed through a Seitz filter and preserved at ice-box temperature under vaseline seal in the special peptone broth described. On the ninth day of preservation a volunteer was inoculated intranasally and within 24 hours developed typical symptoms of the common cold. As soon as the signs of the disease were well-marked, naso-pharyngeal washings were prepared in the usual manner and preserved for 5 days at ice-box temperature. After this interval a second volunteer was inoculated who also developed symptoms of a cold within the usual incubation period. Naso-pharyngeal washings were again prepared, filtered through a Seitz filter and concentrated by vacuum distillation. After 10 days' preservation at ice-box temperature a third volunteer was inoculated. This individual developed a cold of unusual severity, possibly indicating an accession of virulence by the virus. Naso-pharyngeal washings were prepared from this individual after the usual interval, filtered through a Seitz filter, concentrated 7 times by vacuum distillation and preserved for 5 days at ice-box temperature. After the lapse of this interval 0.25 cc. of the concentrated material was inoculated

[3] Foster, G. B., Jr., *J. Am. Med. Assn.*, 1916, **66**, 1180; *J. Infect. Dis.*, 1917, **21**, 451.

[4] Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

[5] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

† Especially prepared peptone obtained through the kindness of Dr. René Dubos of the Hospital of the Rockefeller Institute.

into tissue medium of the nature described. The culture was incubated for 5 days at 37°C. At the end of this time a human volunteer was inoculated and within the usual incubation period developed a cold with symptoms of rather a mild character. From this time on the material was carried in tissue medium and transferred at intervals of from 3 to 9 days. We belive the preferable interval between transfers to be 4 days. From time to time cultures were chosen for the inoculation of experimental subjects. The third culture representing a dilution of the original material of approximately 1-2000 was inoculated into a chimpanzee. This animal developed symptoms of a cold after an incubation period of 4½ days. An uninoculated companion animal kept in the same cage developed symptoms of a cold at the same time. The sixth culture, representing a dilution of the original material of approximately 1-2,000,000, was inoculated into 3 human volunteers. Of these, 2 developed after 24 hours typical colds of moderate severity. The tenth culture, representing a dilution of the original material of approximately 1-20,000,000,000 was inoculated into 3 human volunteers. Of these one developed such mild symptoms of respiratory infection as to be considered doubtful. The twelfth culture representing a dilution of approximately 1-2 trillion was inoculated into 3 human volunteers, and of these one developed a cold with mild symptoms. The fifteenth culture, representing a dilution of approximately 1-2 quadrillion, was inoculated into 3 human volunteers. Of these 2 developed colds of unusually severe symptoms, complete nasal obstruction, frontal headache, malaise, loss of appetite, nasal discharge, and pronounced cough. Naso-pharyngeal washings were prepared from these 2 individuals, filtered through a Seitz filter and concentrated by vacuum distillation. Two human volunteers were inoculated after a short interval with these concentrated filtrates and within 24 hours both developed colds, one with mild symptoms and the other with the typical features of a head cold.

The evidence presented by these experiments indicates that the virus of the common cold survives for a considerable length of time after removal from the human naso-pharynx and that the agent reproduces itself *in vitro* when cultivated in suitable tissue medium. The total duration of life of the culture virus from the time of removal from the human subject has been 74 days. The final dilution of the original material is so great as to rule out conclusively the presence of any significant amount of the original virus. Controls of the constituents of the medium and of the tissue medium have been negative. In one instance when a volunteer received active cul-

ture heated for one-half hour at 80°C. there appeared symptoms of upper respiratory irritation which lasted for less than 24 hours. We realize the difficulty of a final judgment concerning the successful cultivation of an invisible agent and simply present the facts as we have observed them.

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Relationship of Sera and Spinal Fluids to Agglutination and Flocculation by Dyes.

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It has been known for a long time[1] that human red blood cells and bacteria could be agglutinated by solution of basic dyes. In addition M. Gutman[2] showed that whole serum could be flocculated by the same dyes. In our experiments we found that these phenomena were much more complex.

The human red blood cells used in the experiments were washed 3 times in normal saline (0.85%) in order to free them from all traces of serum. A 2% suspension of red blood cells was used. Whenever human serum was used in conjunction with the red blood cells the same types were employed in order to avoid agglutination of the cells by the serum. The standard dye used was a gentian violet solution 1:2500 in normal saline. The ingredients were mixed in equal amounts in narrow glass tubes by thorough shaking. Agglutination and flocculation could easily be observed macroscopically. For microscopic examination drops of the same ingredients were placed on glass slides and mixed with glass rods.

Results. 1. *Flocculation of human sera:* The flocculation of whole human serum by basic dyes noted by Gutman takes place only if relatively strong solutions are added. (*e. g.*, ½-1% aqueous solutions of safranin or gentian violet). But by using weaker solutions different results were obtained. Whole human serum, or serum diluted with normal saline (0.85%) up to 1:6 is not flocculated by our standard solution of gentian violet. Flocculation here first occurs with a serum diluted 1:8 and increases in strength with higher dilutions of sera. In dilutions of serum of 1:64 floccula-

[1] Brossa, G. A., *Z. f. Immunitätsforsch.*, 1923, **37**, 221.

[2] Gutman, M., *Centralbl. f. Bakt.*, I Abt., Orig., 1928, **56**, 68.

tion decreases, and it does not occur when the serum is diluted beyond 1:128.

2. *Agglutination of human red blood cells and bacteria.* In accordance with the work of Gutman and others we found that solutions of basic dyes agglutinate red blood cells and bacteria.

3. *Protective power of human serum.* Whole human serum added to a 2% suspension of human red blood cells or bacteria and thoroughly mixed with them acted as a protective and prevented their agglutination upon the subsequent addition of the gentian violet solution. The serum exerted this protective power in dilutions up to 1:64. In higher dilutions the serum no longer protected and agglutination occurred as if no serum were added.

4. *Desagglutination by serum.* A 2% suspension of human red blood cells was agglutinated by our solution of gentian violet. Equal amounts of whole human serum, or serum diluted up to 1:8 added to the cells agglutinated by the dye caused prompt desagglutination.

5. *Flocculation of spinal fluids.* Our solution of gentian violet added to undiluted spinal fluid or its dilutions up to 1:5 caused their flocculation. In dilutions higher than 1:5 flocculation decreases and does not occur when the spinal fluid is diluted beyond 1:10.

6. *Protective power of spinal fluid.* Undiluted spinal fluid added to a 2% suspension of human red blood cells or to bacteria protects them against agglutination upon subsequent addition of the gentian violet solution.

There are individual variations in the reactivity of human red blood cells, sera and spinal fluids towards dyes. We are now carrying on further experiments to determine whether there are any differences produced by pathological conditions in the human body.

5399

Demonstration of a Tumor-Inhibiting Substance in Filtrate of Rous Chicken Sarcoma and in Normal Chicken Sera.

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In a previous communication[1] we reported that the active agent

[1] Sittenfield, M. J., and Johnson, B. A., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 206.

in the Rous chicken sarcoma filtrate could be concentrated and partially purified by adjusting the filtrate, normally about pH 7.2, to a reaction of pH 4. When brought to this reaction with a phthalate buffer, the tumor-producing agent is carried down with the precipitated portion. When this precipitate is extracted at pH 8, the carcinogenic agent is not only set free, but the activity of the extract seems definitely greater than that of the original filtrate.

To account for the increased activity of the first extract at pH 8, several possibilities suggested themselves; among others, that the active agent in the filtrate may be in combination with substances that restrain its activity to some extent, and that in the process of precipitation by the method described above, there is brought about dissociation between the restraining or protective agent and the tumor-producing element. If true, this would explain why the extract of the first precipitate is considerably stronger than the filtrate from which it was prepared.

To test for the presence of a protective agent, we examined the supernatant fluid obtained after precipitating at pH 4 the active agent from a 10% filtrate. After centrifuging, the supernatant fluid was decanted, neutralized and then concentrated *in vacuo* at room temperature to ½ its original volume. Two cubic centimeters of this concentrated supernatant fluid were now mixed with 0.5 cc. of the original filtrate, containing approximately 2 infective doses, and allowed to stand for 30-40 minutes prior to injection. Chicks, 10-14 days old, were used in all the experiments, for they have been found to give more uniform results. Of 80 chickens inoculated with this mixture, 75% failed to develop tumors, while tumors developed in all the controls which had received this amount of filtrate alone. It was found that smaller doses of supernatant fluid were ineffective, as they gave protection only in rare instances.

In the 25% which developed tumors, the inhibitory effect of the supernatant fluid was shown by the fact that the appearance of the tumors was delayed, they grew less rapidly, did not attain their usual size, and in one or 2 instances receded. When 8 or 10 times the required dose of the active agent is used, with a proportionate increase in the amount of supernatant fluid, no inhibitory effect is observed. With a very active extract of a pH 4 precipitate, increased amounts of the concentrated supernatant fluid must be used, since we found that 2 cc. will not neutralize more than approximately 2 infective doses.

In other words, the first precipitation had brought about a separation between the active agent now held in the precipitate and a pro-

tective fraction which remains behind in the first supernatant fluid. It seemed probable that this protective substance would be present only in the supernatant fluid from the first precipitation at pH 4, and this was found to be true, for the supernatant fluids from the second and subsequent precipitations proved to be non-inhibitory.

By half saturating the supernatant fluid with ammonium sulphate and then dialyzing against running water, it was found that the protective substance was confined to the globulin fraction. In no instance was an inhibiting action obtained with the albumen fraction.

In view of the observations of Rous[2] and Fischer[3] that antisera will protect against inoculations with the free virus but not against the tumor cells, experiments were made to determine if this inhibiting action of the supernatant fluid would be effective in preventing the growth of tumor fragments. Experiments were conducted similar to those described with the filtrate but in no instance were we able to find that the inhibiting substance was effective in preventing the growth of the living tumor cells. These observations are in line with the results obtained by others who have worked with filterable viruses, who claim that immune bodies can be obtained that are effective against the virus outside the cells, but ineffective once the cells are infected.

These observations on the protective substances in the tumor filtrate suggest the probable presence of similar substances in the blood, as claimed by Fischer[3] who, however, does not describe very clearly his methods of demonstrating the presence of the inhibiting action of normal sera. Andrewes[4] and Mottram,[5] using small doses of serum, were unable to demonstrate neutralizing substances in normal chicken sera, but did find them in chickens bearing slow growing tumors. We tested the protective action of the sera of normal and tumor-bearing chickens against the active agent in the Rous chicken filtrate, and found that the serum of normal chickens was protective if used in amounts of approximately 2 cc. to 4 cc. against one or two infective doses. In testing the serum of a chicken partially protected by the injection of a mixture of 2 cc. of concentrated supernatant fluid and 0.5 cc. filtrate, in which the tumor appeared after 19 days and grew very slowly, we found its inhibitory effect was distinctly greater than that of sera of normal chickens.

[2] Rous, Peyton, *J. Exp. Med.*, 1913, **18**, 416.

[3] Fiseher, Albert, *Zeits. m. Krebsforschung*, 1926, **24**, 580.

[4] Andrewes, C. H., *J. Path. and Bact.*, 1931, **34**, 91.

[5] Mottram, J. C., *Brit. J. Exp. Path.*, 1929, **9**, 147.

This chicken was bled 36 days after the inoculation. There are indications that the sera of tumor-bearing fowls in the terminal stages of the disease are not protective to the same degree, but this point is now under investigation. Here, too, as in the chicken filtrate, the protective substance seems to be associated with the globulin fraction. The failure of Andrewes and Mottram to find neutralizing substances in the sera of normal chickens is probably due to the small doses used. In our experience, rarely was a serum active in amounts of less than 2 cc. to 4 cc.

It will probably be found that this protective mechanism is not specific in its action, and that it is equally effective against the filtrates of other filterable chicken tumors. The development of metastases probably depends, as is customarily believed, upon the migration of live cells, for the active agent liberated by the breaking down of tumor cells is probably neutralized by the protective substances in the plasma.

5400

The Gastric Hunger Mechanism. II. The Effect of Diet.

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Balloon tracings of the insulin gastric hypermotility cannot be distinguished by inspection either from the type III hunger contractions, as classified by Carlson,[1] or from the post-feeding tracings described by Mulinos.[2] They can be readily identified, as follows: The post-feeding tracings are uninfluenced by food, or by the intravenous injections of glucose; the hunger type III is depressed by food, but not by glucose intravenously; the post-insulin hypoglucemic hyperactivity is abolished by raising the blood sugar, and removing the hypoglucemia. They are all abolished by adequate doses of atropine.[2]

Two series of 4 female dogs, with gastric fistulae, were studied for 6 to 18 months. The dogs were trained to lie quietly, while the gastric contractions were being recorded by the balloon method described elsewhere,[3] using a chloroform manometer. The insulin

[1] Carlson, A. J., *Am. J. Physiol.*, 1912, **31**, 151.
[2] Mulinos, M. G., *Am. J. Physiol.*, 1927, **83**, 115.
[3] Mulinos, M. G., *Am. J. Physiol.*, 1926, **77**, 158.

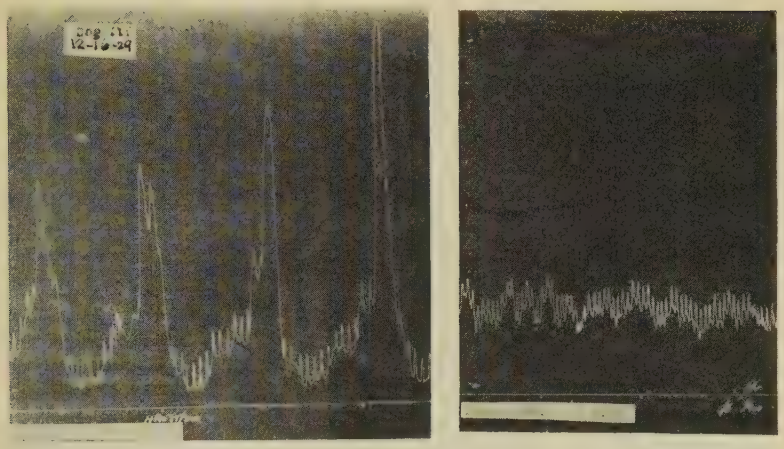

FIG. 1.
Dog balloon record of gastric motility. Gastric response to insulin hypoglucemia after 10 months on the deficient diet. (a) Normal type I contractions. (b) 1 hour after insulin, 2 units per kilo.

was injected subcutaneously or intravenously. No tracings were made within 12 hours of a feeding; the usual time was 18 to 20 hours.

In the first series, insulin hypoglucemia failed to evoke its characteristic gastric hyperactivity. Instead, it produced only an ill-defined undulatory variation in tonus (Fig. 1). The animals of this series were on a diet that consisted of one part of boiled, chopped beef and 2 parts of bread. Although they ate the whole of the portion offered each day, the dogs failed to gain in weight, and occasionally lost a little. It seemed probable that the diet was incomplete, and various modifications were tried. *The gastric response to insulin hypoglucemia returned when there was added to the diet canned vegetable soup.*

The second series of 4 animals had been kept on the above meat-bread-vegetable soup diet for several weeks. These animals gave the usual gastric hypermotility to insulin hypoglucemia. The dogs were then put on the diet consisting of bread and lean meat exclusively, as a control to the first series. Within 1 to 3 months of the change in diet, the gastric hypermotility response to insulin hypoglucemia gradually weakened, and eventually was lost. (Fig. 1.) Along with the decreased gastric response, it was found that the amount of insulin necessary to shock had to be increased by several fold (see note under Fig. 2).

Neither the source of the insulin, nor the oestral status of the animal, nor the salt content of the diet influenced the gastric re-

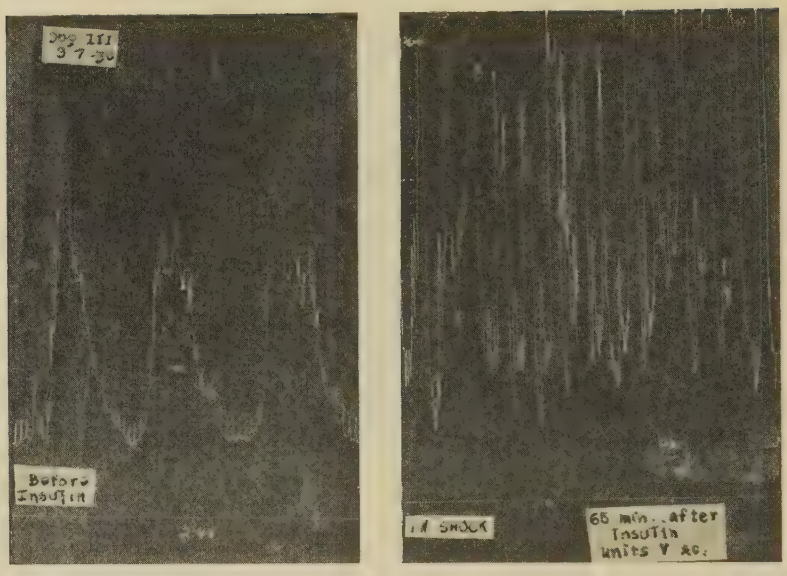

FIG. 2.
Same dog 3 months later, 6 weeks after the addition of a cake of yeast to the daily diet. (a) Normal type I contractions. (b) 1 hour after insulin, units 0.5 per kilo. Compare with Fig. 1 (b). Note the difference in the dose of insulin administered in each case.

sponse to insulin hypoglucemia. However, a week to 10 days after a cake of yeast was added to the daily diet, the stomach began to respond to insulin hypoglucemia by an increase in tonus and activity (Fig. 2).

The insulin necessary to produce shock became progressively less, until the dose was reached to which the dogs had been sensitive before the institution of the special diet. For most of the dogs, the tolerance increased from about 1 unit, on a complete diet, to 2 or 2.5 units per kilo on a bread-meat diet. It was also noted that as the dose of insulin necessary to produce shock increased, the preshock symptoms of salivation, restlessness, twitching and weakness were seldom followed by convulsions; after the addition of the yeast, small doses of insulin frequently led to unheralded convulsions, which required large doses of glucose to suppress.

Insulin, when injected intravenously in from 5 to 20 units (0.25 to 1.0 cc. of U xx) causes a transient but complete inhibition of the gastric hunger contractions, and lowers the gastric tonus. The depression lasts from 5 to 30 minutes, and is often followed by gastric hyperactivity of the hypoglucemic type. The insulins tried were those of Lilly and Mulford. A 0.1% phenol solution (insulin preservative) injected did not have the same effect. This phe-

nomenon was first observed by Simici, Guirea and Dimitriu[4] for man. Templeton and Quigley,[5] however, found that intravenous injections of insulin do not affect the main part of the stomach, but do depress a Heidenhain pouch of that organ.

Conclusions. (1) The response of the stomach to insulin hypoglucemia by hypermotility is dependent upon the presence in the diet of a sufficient amount of a yeast or vegetable factor, probably vitamin B. (2) A diet lacking this factor increases the resistance of the animal to the hypoglucemic effects of insulin, and to the convulsive effects of hypoglucemia. (3) The observation of Simici, Guirea and Dimitriu,[4] that insulin injected intravenously depresses the stomach of man, is confirmed for the dog.

5401

Further Note on the Enumeration of Blood Platelets and Red Blood Cells.

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In a previous communication,[1] a new method of blood platelet and red cell enumeration was proposed in which Ringer-Heparin solution was substituted for Hayem's solution as the diluent. In order to test the preliminary contention that the red blood cells may be counted in the Ringer-Heparin solution as accurately as with Hayem's solution, 75 parallel counts have since been made within 6 hours after the blood was taken. These included 24 counts upon 8 normal and pathological rabbits, 6 counts upon 1 normal and 2 anemic dogs, and 45 counts upon 31 normal and diseased humans, no material being omitted. The means of these parallel determinations are as follows: (1) Hayem's solution: 5,109,700 red blood cells per cmm. (2) Ringer-Heparin solution: 5,116,200 red blood cells per cmm. The standard error of the mean for the 75 counts was approximately the same in each case, that is, 175,000 cells. It may be concluded, therefore, that both methods gave identical results. Hemolysis or fading of the red blood cells with the use of

[4] Simici, Guirea and Dimitriu, *Arch. Malad. Appar. Digest et Malad. Nutrition*, 1927, **17**, 17.

[5] Templeton and Quigley, *Am. J. Physiol.*, 1930, **91**, 467.

[1] Casey, A. E., and Helmer, O. M., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 655.

the Ringer-Heparin solution did not occur in any instance either in the pipette or on the counting chamber.

Other useful points of technique noted since the first report include: (1) autoclaving of the stock Ringer's solution; (2) reduction of the $NaHCO_3$ in the standard Ringer's solution from 0.10 gm. to 0.05 gm. per 1000 cc.; (3) refrigeration of pipettes of diluted blood for delayed counting; (4) reduction of the amount of heparin used for blood counted within 3 hours after taking; (5) the necessity of a thorough mixture through vigorous shaking of the pipettes by hand or machine. It should be pointed out that Hayem's and other fixative solutions should not be used in pipettes employed for Ringer-Heparin solution since hemolysis may occur even after several thorough washings. However, if only Ringer-Heparin solution is used in pipettes, no difficulties with hemolysis will be encountered. Facility in counting is best secured with a fairly strong artificial light, using the low power lens, and keeping the substage shutter closed. The haemocytometer should be so placed that the entire fine ruling may be seen as one field. After standing for at least 15 minutes, to and fro movement of the fine adjustment of the microscope reveals the blood platelets as minute black refractile particles, discrete and distinct. No difficulty in their enumeration has been encountered.

Continued use of the method reaffirms its practicability, speed, and accuracy in the counting of both red blood cells and blood platelets.

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A Delimitation of the Central Nervous Mechanism Involved in Reflex Hyperglycemia.

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Claude Bernard's sugar puncture experiments furnished some evidence of the existence of a bulbar control of carbohydrate mobilization, but efforts to locate a specific center responsible for the piqûre diabetes have been rather inconclusive. Furthermore, temporary hyperglycemia and glycosuria often follow *any* injury to the brain. On the other hand, numerous investigators have shown that specific centers within the diencephalon may play some part in

the regulation of carbohydrate metabolism. Cannon and Rapport[1] produced additional evidence of a localized central control of the blood sugar level by locating a center in the medulla which is responsible for the reflex activity of the adrenal glands. Griffith[2] studied the various factors involved in a reflex hyperglycemia and found that the liver and the adrenal glands are chiefly responsible for the rise. Bard[3] suggested that a comparison of the reflex rises in blood sugar, obtained from intact animals and from animals after various parts of the central nervous system had been removed, might furnish additional information concerning the location of the centers controlling carbohydrate mobilization.

Before an attempt could be made to detect abnormalities in the reflex hyperglycemic reactions, control experiments had to be performed in which the central nervous system had been left intact. Twenty-six such experiments were performed: 10 of the animals were anesthetized by chloralose (0.1 gm. per kilo) administered by mouth; 10 by intravenously injected sodium barbital (0.28 gm. per kilo) and 6 by intraperitoneally injected sodium barbital (0.3 gm. per kilo). Comparable results were obtained in all cases and therefore sodium barbital, injected intravenously, was generally employed in subsequent experiments. Three blood samples were taken at half-hour intervals before stimulation; after a 3-minute period of interrupted (10 sec. on, 5 sec. off, 10 sec. on, —) tetanic stimulation of the right brachial nerve, blood samples were taken at intervals of one minute, 10 minutes, 30 minutes, one hour, and sometimes 2 hours. In all instances the strength of the stimulus was the same. Folin's[4] method of blood sugar assay was employed, 0.5 cc. of blood being drawn for each sample. Well nourished cats which had not been fed for 24 hours were used.

Reflex rises in blood sugar were studied in 10 thalamic animals, 26 decerebrate animals (10 under chloralose, 10 under sodium barbital injected intravenously, and 6 under sodium barbital injected intraperitoneally), and 10 animals in which the brain stem was divided by a section passing from a point a few millimeters posterior to the *corpora quadrigemina,* dorsally, to the posterior border of the pons ventrally. Ten of the decerebrate preparations had first been used as controls, thus making possible a comparison of the reflex rises obtained from the same animals before and after decerebra-

[1] Cannon, W. B., and Rapport, D., *Am. J. Physiol.*, 1921, **58**, 338.

[2] Griffith, F. R., *Am. J. Physiol.*, 1923, **66**, 618.

[3] Bard, P., *Arch. Neurol. and Psychiat.*, 1929, **22**, 230.

[4] Folin, O., *J. Biol. Chem.*, 1929, **82**, 83.

tion. Records of arterial pressure and heart rate were taken throughout each experiment in order to ascertain whether or not the animal was in good condition. After the transection had been performed an hour or more was allowed to elapse before the stimulation. This permitted the blood sugar to return to an approximately normal level. The reflex rises in blood sugar obtained in these transected animals were practically equal to those evoked in cats with the central nervous system intact (See Table).

TABLE I.

Reflex Rises in Blood Sugar Level Obtained After Transection of the Brain Stem at Various Levels.

Type of Preparation	No. of Animals	Aver. Blood Sugar Level Before stimulation.	Aver. rise in mg. of Sugar per 100 cc. of blood.	Aver. Rise in %.	Deviation from the Rise Obtained in Control Experiments.
Controls	26	130	24	19	
Thalamic	10	147	26	18	—1%
Decerebrate	26	138	29	21	+3%
Section anterior to *Brachium Pontis*	10	164	26	16	—3%
Section posterior to *Brachium Pontis*	5	140	—1	—0.7	No rise
Decapitate	15	154	—15	—9	No rise

Decapitate animals, on the other hand, did not respond to afferent stimulation with an increase in the blood sugar concentration, even though slight rises in arterial pressure and heart rate were obtained. Nor was a reflex hyperglycemia obtained if the medulla oblongata had been transected posterior to the anterior border of the *brachium pontis*. Therefore, it can be concluded that there is a mechanism located in the medulla oblongata, in the region of the vasomotor center, which is essential to a normal rise in blood sugar concentration when an afferent nerve is stimulated. This does not indicate that higher centers may not influence carbohydrate mobilization, but it does indicate that they are not essential to a normal reflex hyperglycemia. The results obtained are summarized in the accompanying table.

5403

Action of Viosterol and Parathormone in Thyroparathyroidectomized Dogs.

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(Introduced by George R. Cowgill.)

From the Departments of Surgery and Physiological Chemistry, Yale University.

The daily intake, output, and blood serum level of calcium and phosphorus, together with the muscular responses to electrical stimuli, were studied in 2 adult, female dogs before and after thyroparathyroidectomy. An extensive dissection was done in order to insure removal of all parathyroid tissue.

Throughout the investigation a basal ration prepared according to the technique of Cowgill[1] was employed. The *postoperative* period was divided into 3 parts. In the first, the animals received subcutaneous injections of parathormone.† In the second, viosterol‡ was administered by mouth. Because of its delayed action on calcium metabolism, the viosterol was at first supplemented by parathormone and then was given alone. In the third part, the dogs were allowed to subsist again on the experimental ration without the aid of either parathormone or viosterol.

Results. Phosphorus metabolism will not be discussed in this report. The following discussion of the calcium data from one of the animals serves to illustrate the results obtained.

During the *administration of parathormone,* the curve of urinary calcium rose and then fell, whereas that of the total output (feces essentially) was for the most part unchanged except for a slight final elevation. (See Fig. 1.)

During the *administration of viosterol* the curve of urinary calcium, after an initial lag, rose gradually and steadily, whereas that of the total output (feces essentially) fell to a low and fairly constant level.

In the *posttherapeutic phase* (basal diet alone without either supplement) the curve of urinary calcium continued to rise and then gradually fell in the course of about 2 weeks to the level that prevailed at the beginning of the administration of the viosterol. At first the total output of calcium stayed constant, then abruptly fell

* Seessel Fellow, Yale University, 1929-30.

1 Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 725.

† Donated by the Eli Lilly and Company, Indianapolis, Ind.

‡ Donated by the Mead Johnson and Company, Evansville, Ind.

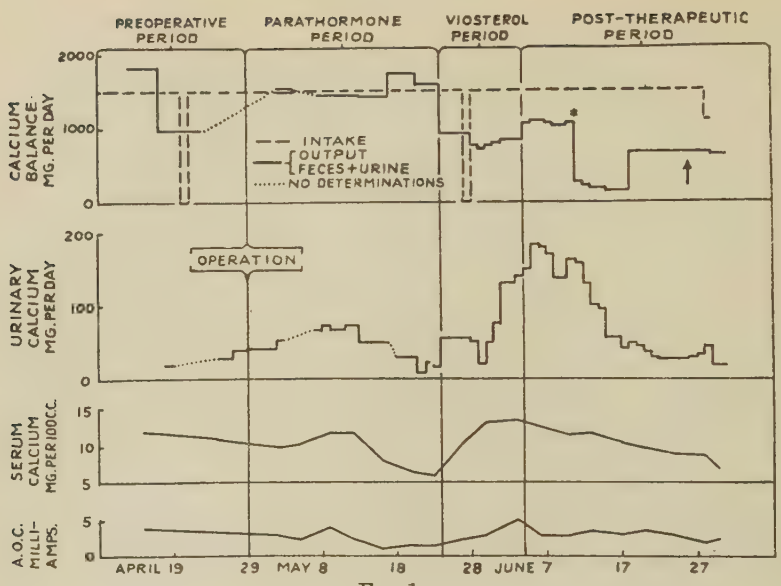

FIG. 1.

to a lower level than had been observed at any time before. It later rose to about two-thirds of the height from which the drop occurred, and it there remained unchanged. During this time (see arrow) the dog under consideration was discovered eating its feces. Consideration of the ash value of the stools and other observations makes it quite probable that coprophagy began at about the time (see asterisk) the marked drop in the fecal output of calcium occurred in the illustrative data here discussed.

It is of interest to point out the parallelism exhibited by the curves of the anodal opening contraction, blood serum calcium and, to a lesser degree, that for the urinary calcium. In the case of the last-named, both the rise and fall were somewhat delayed as compared with the curves for electrical reaction and blood serum calcium.

Conclusions. 1. In thyroparathyroidectomized dogs suitable doses of either viosterol or parathormone will cause a rise in the level of the blood serum calcium, which is remarkably parallel to that of the electrical reaction figures, and, to a lesser extent, that of the urinary calcium excretion values. 2. The elimination of calcium by way of the feces is apparently increased during the administration of parathormone and is decreased during that of viosterol, thus tending to produce a negative calcium balance in the former and a positive one in the latter instance.

5404

Some Effects of Temperature Upon Development of the Oocysts of Coccidia.

E. R. BECKER AND H. B. CROUCH.

From the Zoology Department, Iowa State College.

Pérard[1] has shown that while the oocysts of the rabbit coccidia are extremely resistant to chemicals, they are, on the other hand, quite susceptible to heat. For example, *Eimeria perforans* was rendered incapable of sporulating if kept in an aqueous medium at a temperature of 40°C. for one day. Eighty per cent. of the oocysts were killed within 20 minutes in water at 55°C., within 10 seconds in water at 80°C., and within 5 seconds by boiling water.

In our experiments with *E. perforans* and *E. magna* the temperatures were maintained by an electric thermo-regulator water bath which did not fluctuate much beyond 0.1°C. The oocysts were kept in a 2% aqueous solution of potassium dichromate aerated by a constant succession of bubbles of air which was humidified and equated to the temperature of the bath prior to coming into contact with the culture solution.

At 25°C. about 50% of the oocysts of *E. magna* completed the sporulation process within 84 hours, while *E. perforans* required but 48 hours to attain the same percentage of development. At 33°C. 80% of *E. magna* sporulated within 72 hours, while all of *E. perforans* sporulated within 48 hours. A comparison of both the rapidity of development and the percentage of degeneration at higher and lower temperatures indicates that 33°C. is the optimal temperature for the sporulation of oocysts of both species. At 35° and 36°C. the number of degenerated oocysts after 72 hours varied considerably in the different trials, but in nearly all cases it was at least 50%. Although the developmental process sometimes progressed to the sporoblast stage, we were unable to find any sporozoites in *E. magna* which had been kept at 36°C. for any length of time. At this temperature only 10 to 14% of *E. perforans* completed sporulation, the rest becoming degenerated.

Oocysts of both species kept at temperatures of from 40° to 50°C. inclusive for 10 minutes were capable of further development. In the case of *E. magna,* however, we were able to find only 6 sporulated oocysts in 6 tubes of oocysts which we had attempted to culture after they were subjected to a temperature of 50°C. for 10

[1] Pérard, C., *Ann. Inst. Pasteur,* Paris, 1925, **39**, 505.

minutes. At temperatures of 51°C. and above for 10 minutes the oocysts of both species were rendered incapable of further development. The thermal death point of the undeveloped oocysts of *E. magna* and *E. perforans* in a watery medium with 2% potassium dichromate is, then, 51°C.

TABLE I.

Comparison of Numbers of Oocysts that Developed and Degenerated at Different Temperatures.

E. magna.

C°	Hours	Developed	Degenerated	Arrested Development
		%	%	%
10	325	0.0	100.0	0.0
12	226	0.0	90.0	10.0
25	84	50.2	10.0	39.8
30	84	60.2	20.2	19.6
33	72	80.0	10.0	10.0
35	96	40.6	50.4	9.0
36	132	0.0	100.0	0.0

E. perforans.

C°	Hours	Developed	Degenerated	Arrested Development
		%	%	%
10	60	30.0	10.4	59.6
12	60	50.2	10.0	39.8
25	48	50.0	4.0	46.0
30	48	40.0	4.0	66.0
33	48	100.0	0.0	0.0
36	144	10.0	90.0	0.0

"Arrested development" represents those oöcysts that neither completely developed nor degenerated within the hours listed to the left.

The data obtained in the experiment show that the optimum temperature for the development of *E. magna* and *E. perforans* in a 2% potassium dichromate solution is 33°C., and the thermal death point of the non-sporulated oocysts is surprisingly low, 51°C.

5405

Mobilization of the Inorganic Constituents of the Blood by Vagal Stimulation.*

E. G. LIPOW, C. I. REED AND WILLIS K. WEAVER.

From the Department of Physiology, University of Illinois College of Medicine.

Since it was necessary to carry out a part of this investigation under ether anesthesia, it was first necessary to establish type graphs of the concentrations of Ca, K, P, Na, and of Mg during 2 hours of ether anesthesia. This was done on 20 normal dogs. The re-

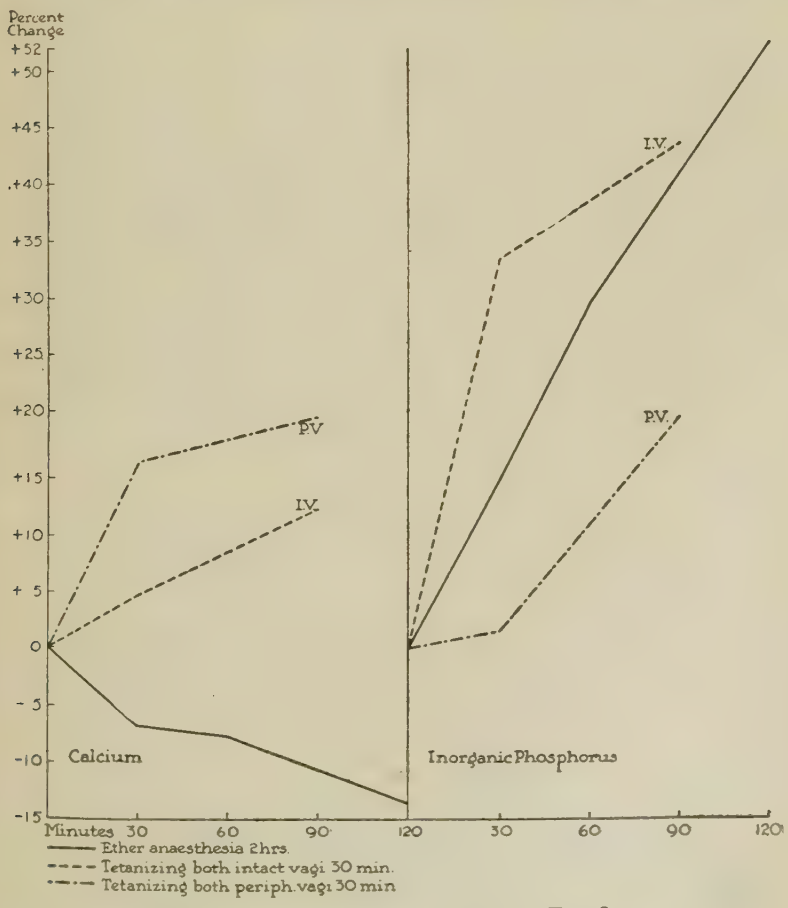

FIG. 1.

FIG. 2.

* This investigation was financed in part by a grant from the Graduate School Research Fund.

sults in general conform well with those reported by other investigators.

In a second series of 13 dogs, anesthesia was induced after a sample of heart blood was taken. Both vagi were then isolated and shielded electrodes from a common inductorium attached. The

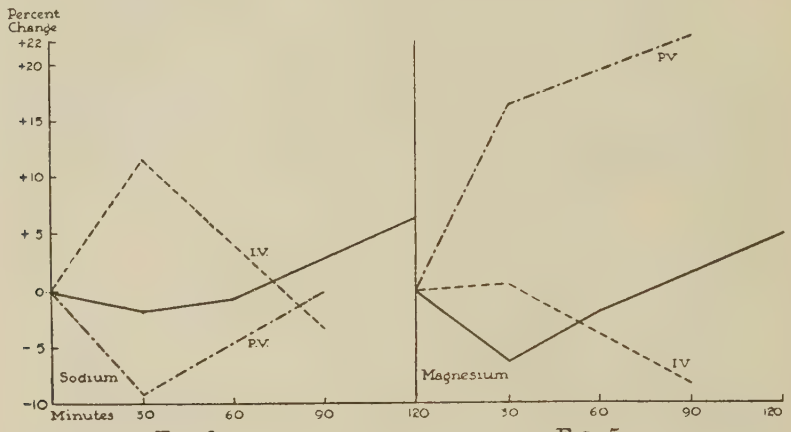

FIG. 3. FIG. 5.

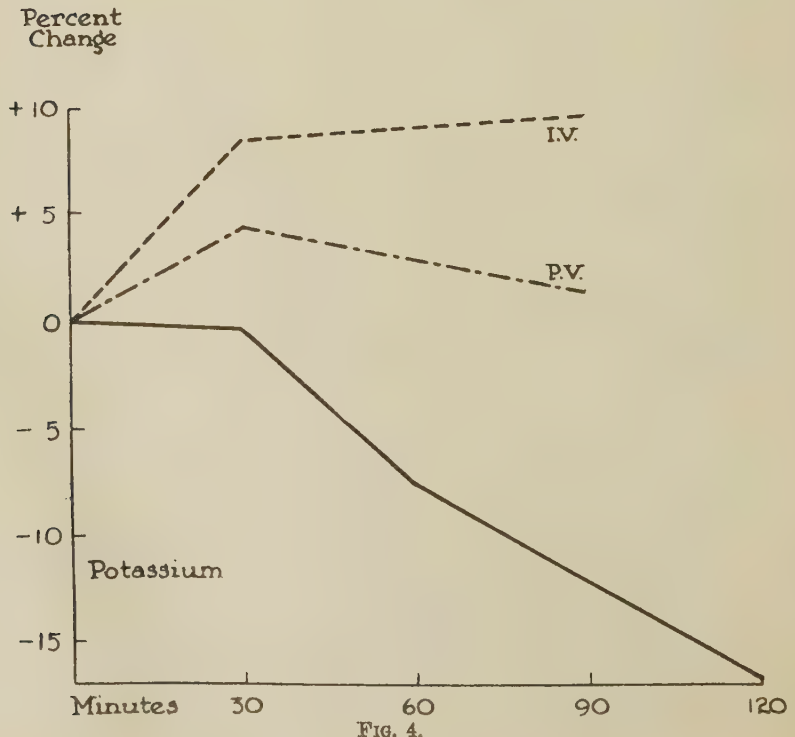

FIG. 4.

intensity of current was adjusted so that neither heart rate nor respiration were markedly affected. In no case was the former decreased more than 5 beats per minute. It is believed that this represented a stimulus of physiological intensity. A tetanizing current so adjusted was applied for 30 minutes after which a second blood sample was drawn, and a third at the end of another hour. In a third series of 8 dogs similar technic was employed except that both vagi were sectioned and the electrodes were applied to the peripheral ends. The composite graphs constructed on a percentage basis are shown in the accompanying figures. With exception of potassium the responses in the individual experiments conformed well with the composite graphs which may, therefore, be considered as type graphs.

It is recognized that the anesthetic is a complicating factor and that these experiments merely demonstrate the modification of the anesthetic graph by vagal stimulation.

The differences observed between peripheral vagal stimulation and intact vagal stimulation may be ascribed to antagonistic reflexes aroused by impulses conveyed centrally from the point of stimulation in the latter group of experiments. A point of some interest is the similarity of all the graphs for calcium to those for potassium.

These experiments demonstrate that, with the possible exception of inorganic phosphorus, the concentration of inorganic salts in the blood may be influenced by the autonomic nervous system, regardless of the immediate mechanism of nervous control.

5406

Experimental Production of Mucous Plugs in the Bronchi of Dogs.*

SELLING BRILL AND A. LINCOLN BROWN. (Introduced by C. D. Leake.)

From the Department of Surgery, Division of Thoracic Surgery, and the Pharmacological Laboratory of the University of California Medical School, San Francisco.

The most commonly accepted explanation for the production of post-operative pulmonary atelectasis is bronchial obstruction, either by a mucous plug or by bronchial secretion. The obstruction of the bronchus or bronchi must be complete so that air can not enter the lung distal to the obstruction. The trapped air or gas in this ob-

* Supported in part by the J. J. and Nettie Mack Foundation.

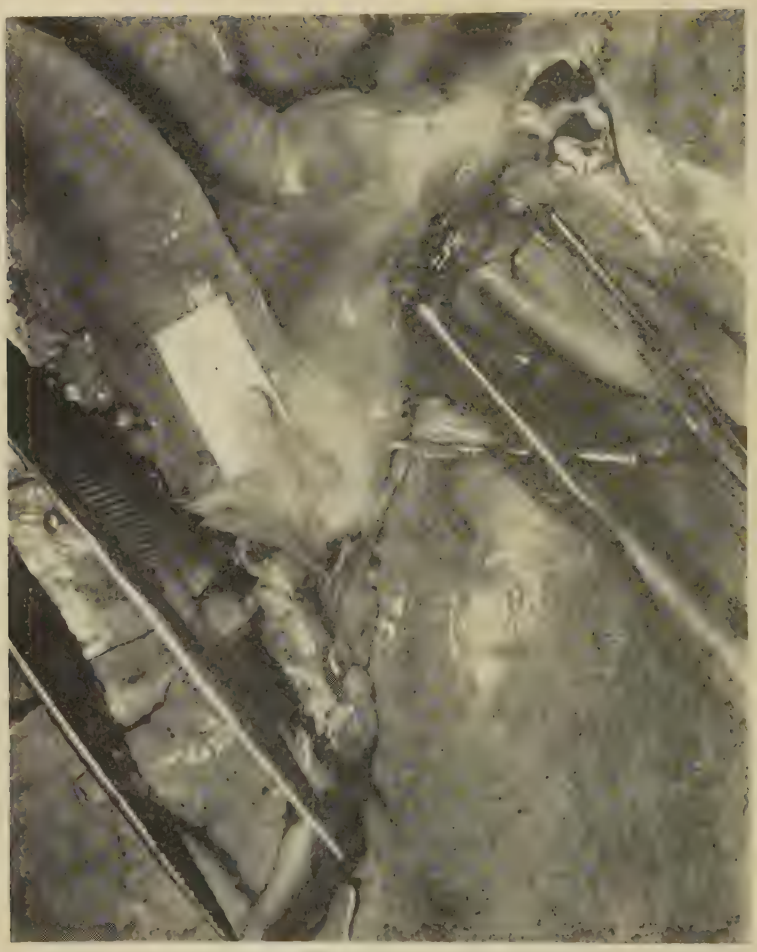

FIG. 1.

Photograph of trachea, bronchi, and lungs of Dog VII, killed 72 hours after introduction of mustard oil into the bronchus of the right lower lobe and 48 hours after plug was seen through the bronchoscope filling that bronchus and extending into right primary bronchus. An idea of the size of the exposed part of the plug can be obtained by comparison with the pins holding the right primary bronchus open.

structed part of the lung is then absorbed by the blood and so the lung collapses or becomes atelectatic.

To study this mechanism, a large variety of foreign bodies have been used to plug the bronchi in dogs such as shot, paper balls, gum arabic,[1] sponges,[2] peas,[2] ligation of a bronchus,[2] rubber balloons,[2]

[1] Mendelsshon, A., *Der Mechanismus der Respiration and Cirkulation*, etc. B. Behrs, Berlin, 1845.

[2] Lichtheim, L., *Arch. f. exp. Path. u. Pharmakol.*, 1878, **10**, 54.

corks,[6] etc. Some investigators[7] have even used tenacious secretions removed from the bronchial tree of a patient with massive atelectasis. It seemed to us that, rather than introduce foreign bodies into the bronchi of dogs, the condition in the human would be simulated more nearly if a mucous plug or tenacious secretion could be produced in the bronchi by irritation. It was found that mustard oil[8] was admirably suited for this purpose.

The dogs were first anesthetized, usually with sodium amytal. Bronchoscopy was performed and the bronchial tree examined to make certain it was normal. A small cotton pledget moistened with mustard oil was then introduced through the bronchoscope into the desired bronchus and held there for 2 or 3 minutes. This area was examined subsequently at frequent intervals through the bronchoscope.

It was found that a bronchial plug of the type shown in the illustration was formed usually in about 24 hours. With one exception there was little reaction in the rest of the bronchial tree. This type of plug was found to extend deep into the finer ramifications and was frequently over an inch in length. There was usually tenacious secretion about the plug. Microscopically the plug consisted either of pure mucous or largely of fibrin and leucocytes. In seven experiments bronchial plugs of the type indicated were successfully produced by the technique described. In one animal a localized bronchial edema occurred at the site of application of the mustard oil. The roentgenographic and pathologic studies on the lungs will be presented in another communication.

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[3] MacCallum, W. G., *Johns Hopkins Hosp. Bull.*, 1908, **19**, 215.

[4] Andrus, W. DeW., *Arch. Surg.*, 1925, **10**, 506.

[5] Coryllos, P. N., and Birnbaum, G. L., *Arch. Surg.* 1928, **16**, 501.

[6] Van Allen, C. M., and Adams, W. E., *Surg. Gynec. and Obst.*, 1930, **50**, 385.

[7] Lee, W. E., Ravdin, I. S., Tucker, G., and Pendergrass, E. P., *Ann. Surg.*, 1928, **88**, 15.

[8] Suggested by Prof. C. D. Leake.

5407

I. Influence of Lactic Acid on Unicellular Organisms.

I. A. PARFENTJEV, W. C. DEVRIENT AND BORIS SOKOLOFF.
(Introduced by Leo Loeb.)

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It is well known that malignant tumors produce a greater amount of lactic acid than the large majority of normal tissues. It appeared to us, therefore, of interest to study the effect of lactic acid on the proliferative activity of different kinds of cells and organisms. We undertook experiments on the influence of lactic acid on the growth of paramecia, yeast, rats, and malignant tumors. We neutralized the C.P. market preparation of lactic acid with sodium hydroxide until the different pH's desired were obtained. Lactic acid solutions were prepared varying between pH 3 and pH 7. In each experiment distilled water acidified with so much hydrochloric acid that the same pH was obtained as in the lactic acid solution was used as control.

In our experiments with paramecia we found that pure lactic acid is very toxic because of its strong hydrogen ion concentration. In a solution of lactic acid 1:5,000 the paramecia died within a few minutes, and even when the solution was weakened to 1:10,000 a high mortality of the paramecia was noted.

Lactic acid in the concentration of 1:1,000 neutralized with sodium hydroxide to pH 7 kills the paramecia within one or 2 days. Weaker solutions of lactic acid such as 1:5,000 at pH 7 begin to inhibit the multiplication of paramecia after some time, and kill these organisms after from 5 to 7 days. This can be seen in the following table.

TABLE I.
Number of Paramecia in 4 Watch Glasses, daily.

Hours Elapsed	24	48	72	96	120	144	168	Average
Control	10	7	7	7	5	8	8	52
With lactic acid	8	8	2	1	2	1	0	22
			pH 6.9		T 28°C.			

This table shows one of our many experiments in which we compared the rate of multiplication in paramecia in hay extract alone (control) and in the same kind of hay extract after the addition of lactic acid in a concentration of 1:5,000.

In each experiment we used 8 watch glasses, 4 of which served

as controls, and 4 of which contained lactic acid. Both of these groups of watch glasses were kept under the same conditions. Each figure in the second and third lines of the table represents the number of paramecia found in the 4 watch glasses. Every day each watch glass was examined and from those which showed an increase in the number of paramecia all save one were removed. In each watch glass, the solution was renewed every day. More than 100 series of experiments of this kind, extending over a period of several months, were carried out.

In another series of experiments we tested the influence of lactic acid on yeast.

TABLE II.
Number of Yeast Cells in 1 cc.

Time in hours	Control	Lactic acid 1:500
0	6,320	6,320
24	6,760	6,520
48	7,200	5,800

pH 3 T 27°C.

Table II shows the rate of multiplication of yeast cells in a nutritive solution of Euler-Svanberg. We compared the rate of multiplication in this nutritive solution (control), and in the same solution with lactic acid 1:500, in each case the acid being neutralized to pH 3 in the Euler-Svanberg solution. In the controls a pH of 3 was obtained through the addition of a small amount of hydrochloric acid. Although the yeast cells tolerated lactic acid better than paramecia, we found that in concentrations of 1:500 and 1:1,000 it likewise inhibits the multiplication of yeast cells. Eighteen series of experiments with yeast cells were carried out; they all gave the same result. The experiment cited in the paper represents one of these 18 experiments.

We may therefore conclude that lactic acid inhibits the multiplication of cells differing so much from each other as paramecia and yeast.

5408

II. Influence of Lactic Acid on Transplanted Tumors.

I. A. PARFENTJEV, W. C. DEVRIENT AND BORIS SOKOLOFF.
(Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine. St. Louis, Missouri.

Continuing our work on the action of lactic acid on various kinds of organisms we carried out a series of experiments on the effect of lactic acid on the growth of transplanted Sarcoma 39 in white rats* and of transplanted Sarcoma Rous in chickens.†

White rats were injected daily with lactic acid previously neutralized with sodium hydroxide to a pH ranging between 5 and 7 with doses of lactic acid ranging between 0.1 gm. and 0.3 gm. per kg. of body weight. These injections were made subcutaneously at a distance from the tumor and they were begun when the Sarcoma had reached the size of 0.5 to 1.0 square centimeters. This series of experiments was carried out with 90 rats having transplanted tumors, 28 of which served as controls. The size of the tumor in each case was measured with a compass and a ruler. The injections of lactic acid neutralized to pH of from 5 to 7 did not produce any ulcerations in rats. The injections of a lactic acid preparation with a pH 3 as well as of solutions of lactic acid in distilled water with a pH below 3 produced ulcerations of the tumors and the overlying skin. At the end of the experiments the control rats had tumors with an average size of 11.2 square centimeters, while those treated with lactic acid had an average size of only 3.0 square centimeters. The percentage of complete disappearance of Sarcoma 39 under these conditions was 50%. In the above mentioned control rats we did not observe spontaneous disappearance of tumors.

Another series of experiments with Sarcoma 39 was carried out in which 3 out of 10 control tumors spontaneously retrogressed and 22 out of 28 treated tumors disappeared.

Another series of experiments with Sarcoma 39 was carried out, chickens bearing Sarcoma Rous were injected with lactic acid. In some cases we obtained an inhibition of the growth of the tumor similar to that observed in the following experiment in which 12

* Through the kindness of Dr. F. C. Wood, Sarcoma 39 was secured from the Institute for Cancer Research of Columbia University in New York City.

† We are indebted for Sarcoma Rous to the kindness of Dr. J. B. Murphy of the Rockefeller Institute.

chickens were used. The 12 chickens were divided into 2 groups of 6 each. Those in the one group were injected with lactic acid which had been neutralized to pH 5. These received daily injections of 0.3 g. of lactic acid per kg. of body weight during a period of 5 weeks. The chickens in the other group were used as controls. After 3 weeks had elapsed all 12 chickens were grafted with Sarcoma Rous. The 6 chickens which had been subjected to preliminary lactic acid injections for 3 weeks were now subjected to the same treatment for an additional 2 weeks. Five weeks after the beginning of the experiment it was observed that the injected chickens had no tumors at all, while the control animals showed tumors of an average size of 1.3 square centimeters. It seems then that injections of lactic acid begun previous to inoculation with tumor and continued over a long period of time will inhibit the growth of Sarcoma Rous.

In the next experiment 6 chickens were used, 3 of which served as controls, all the animals having tumors of about 6 square centimeters average size. Each of the chickens received 6 injections of pure lactic acid in amounts of 0.1 gm. per kg. of body weight. The injections were made directly into the tumor tissue. After 10 days the average size of the tumors in the injected chickens was 11.0 square centimeters while the size of those in the control chickens was 17.0 square centimeters. Neither the injections of pure lactic acid nor those in which the acid had been partly neutralized produced any ulcerations in the chickens.

We may then conclude that though injections of lactic acid were able to inhibit the growth of the transplanted Sarcoma 39 in rats and of Sarcoma Rous in chickens, it remains to be determined through what mechanism this action of lactic acid is produced, and how far similar effects can be accomplished also by the use of other substances.

5409

Influence of Quinone on Oxidation Processes, Proliferative Activity and Longevity of Yeast.

G. LEJHANEC, I. A. PARFENTJEV AND B. SOKOLOFF.
(Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Devrient, Thyssen, and Sokoloff[1] have shown that various substances originate as a result of decomposition of adrenalin, some of which have a specific action on cells. Among these substances is p-quinone which possesses a strong oxidizing power. We determined the influence of p-quinone on the longevity, cell multiplication, and metabolism of yeast cells.

We measured the influence of quinone on the oxygen consumption of yeast cells, using the differential manometer of Barcroft-Drastich.[2] The yeast was either in the form of a 36% suspension in water, or a 5% suspension in nutritive solution of Euler and Svanberg. We found that p-quinone in the concentration of 1:500,000 increases the oxygen consumption of yeast cells and that this increase is relatively higher when the yeast cells are suspended in the nutritive solution.

We investigated the influence of quinone on the multiplication and longevity of the yeast cells. For the study on proliferative activity we used yeast suspended in nutritive solution, in which the conditions for cell multiplication are optimal, and the effect of quinone on longevity was studied when cell multiplication was minimal.

In the first experiments we determined the influence of quinone in the concentration of 1:500,000, which according to Kisch and Leibowitz,[3] is optimal as far as the effect on the stimulation of oxygen consumption of normal kidney tissue is concerned. We found a diminution in the number of yeast cells when compared with controls. This diminution was manifest in the experiments in which cells multiplied as well as in those in which they failed to multiply.

In the second series of experiments the effects of different quinone concentrations from 10^{-6} to 10^{-4} were examined. In general,

1 Devrient, Wm., Thyssen, St., and Sokoloff, B., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 80.

2 Drastich, L., *Biochem. Z.*, 1927, **188**.

3 Kisch, B., and Leibowitz, J., *Biochem. Z.*, 215.

quinone in the concentration of 10^{-6} and lower has no effect on the multiplication of yeast cells. But with the increase in the concentration to 10^{-4} the depressing effect on the multiplication of yeast cells increases. This effect is most marked on the first day, becoming subsequently less marked because yeast cells are able to reduce quinone to hydroquinone and quinhydrone (Luers[4]), substances which according to the experiments of Hinteregger[5] with frog muscles are not so toxic as quinone.

Our experiments have shown that quinone in a very dilute solution (1:500,000) tends to increase the oxygen consumption of yeast cells and at the same time to shorten the life of these cells and to inhibit their multiplication.

It is of great interest to note that quinone exerts its influence in concentrations so low that it may be considered as oligodynamic.

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[4] Luers, *Biochem. Z.*, 179.

[5] Hinteregger, *Archiv. f. Exp. Pharm. and Pathol.*, 1930, 156.

Minnesota Section.

University of Minnesota, January 28, 1931.

5410

Absorption of Hydrokollag from the Obstructed Bowel.*

H. A. CARLSON, H. J. DVORAK, F. W. LYNCH AND O. H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

A colloidal suspension of graphite known as Hydrokollag has been employed for the intravital injection of capillaries[1] and for the study of phagocytic cells. When this preparation is injected into the circulation, the black graphite particles are taken up by the reticulo-endothelial cells and may be readily demonstrated within these cells under the microscope. The object of our experiments was to determine whether in bowel obstruction the intestinal mucosa was sufficiently altered to permit of absorption of the graphite. Absorption does not occur in the normally unobstructed bowel. Herrmann and Higgins[2] have already reported absorption of Hydrokollag from the obstructed colon in cases in which there was ulceration of the mucosa.

Three forms of intestinal obstruction were studied in this series of experiments, *viz.*, simple, strangulation, and loop obstruction. Simple obstruction at different levels was produced by dividing the bowel and turning in both ends after having placed Hydrokollag in the proximal loop. Strangulation obstruction was accomplished by tying off a segment of bowel together with its blood supply. Hydrokollag was injected into the lumen of the strangulated segment. In loop obstruction the bowel was divided in 2 places, the 2 ends of the isolated loop turned in after Hydrokollag had been introduced into the loop. Intestinal continuity was re-established by anastomosis of the loops of bowel proximal and distal to the ob-

* This work was supported by Grant 188 allowed by the Committee on Scientific Research of the American Medical Association.

[1] Drinker, C. K., and Churchill, E. D., *Proc. Roy. Soc. London*, 1927, **101**, 462.

[2] Herrmann, S. F., and Higgins, G. M., *Am. J. Med. Sci.*, 1930, **179**, 36.

structed loop. The loop obstruction may be regarded as a form of strangulation obstruction. The amounts of Hydrokollag placed in the bowel in all types of obstruction varied from 20 to 50 cc. Usually 50 cc. was introduced into the lumen of the bowel.

When an animal (dog or rabbit) died or was sacrificed, specimens of liver, spleen and sometimes of the abdominal lymph nodes, kidney or lung were obtained. Eventually paraffin sections were prepared and stained with hematoxylin and eosin and examined microscopically for the presence of graphite particles. Formalin was first employed as the fixative but the not infrequent presence of formalin pigment proved to be a source of confusion. Later Zenker's solution was used for fixing the tissues. However, even when the animals were sacrificed and the tissues immediately fixed in Zenker's solution, black precipitate could be observed on some of the slides when studied under the microscope. The differentiation between graphite and extraneous pigment was in some instances very difficult if not actually impossible. The results reported here include only those experiments in which the tissues were fixed in Zenker's solution shortly after the death of the animal. In most instances the animals were sacrificed and specimens of tissue fixed at once.

The series included 29 dogs and 5 rabbits with intestinal obstruction who died or were sacrificed 1 to 10 days after the performance of the obstructing operation. In 2 additional dogs used as controls, 10 cc. of Hydrokollag was injected into the mesenteric veins. They were sacrificed 2 and 6 days after injecting Hydrokollag into the portal circulation.

Experience with the intraportal injections revealed that the presence of graphite particles within the Kupfer cells of the liver was the most reliable criterion of the presence of graphite in the portal circulation. Therefore, only when black particles appeared to be definitely within these cells were they regarded as absorbed graphite particles.

Results. The accompanying table records the presence or absence of graphite in the liver and abdominal lymph nodes. The tabulated results clearly indicate that under ordinary circumstances Hydrokollag is not absorbed from the obstructed intestine either by way of the lymphatics or the portal circulation. Only in the instance of a loop obstruction which became ulcerated and perforated did any Hydrokollag reach the liver; as some graphite was spilled into the peritoneal cavity as a result of the perforation it may have reached the circulation after having been absorbed from the peritoneal cavity.

TABLE I.

Indicating the presence or absence of graphite particles in the liver and lymph nodes.

	Liver			Lymph Nodes		
	Number	Present	Absent	Number	Present	Absent
Simple obstruction						
Duodenum (dog)	3	0	3	3	0	3
Ileum (dog)	13	0	13	6	1	5
Ileum (rabbit)	5	0	5	—	—	—
Colon (dog)	6	0	6	2	0	2
Loop obstruction	6	1	5	1	0	1
Strangulation obstruction	1	0	1	—	—	—
Controls						
Intraperitoneal injections of Hydrokollag	2	2	0	1	0	1

A very small amount of pigment interpreted as graphite was found in one abdominal lymph node in the case of a dog with simple obstruction of the terminal ileum. In this experiment there was a peritonitis due to slight leakage from the inverted end of the obstructed bowel. In this case, however, the evidence is not complete as to whether the graphite was absorbed from the intestinal lumen or from the peritoneal cavity.

Gross and microscopic examination of the intestinal wall failed to reveal evidence of ulceration in the cases of simple obstruction. The results reported here are, therefore, in accordance with those of Herrmann and Higgins, who failed to note any absorption in the absence of mucosal ulceration when Hydrokollag was placed in the obstructed colon of dogs.

Conclusions. No evidence was obtained to indicate that in high, mid, or low obstruction of the simple type that Hydrokollag was absorbed. These results lend no support to the "absorption of toxins" theory of the cause of death in simple obstructions in which the bowel is viable.

5411

Hunger Sensations in a Patient After Total Gastrectomy.

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The contraction of the empty stomach is regarded as an essential element in the sensation of hunger in man and animals on the basis of numerous experimental studies, although the possibility of other factors contributing to hunger has been considered. Carlson,[1] and Ivy and Vloedman[2] expressed interest in the question of hunger following removal of the stomach but were unable to find in the literature any reports based upon complete gastric excision.

An opportunity to investigate the question of hunger sensation in man following removal of the stomach was afforded by a patient upon whom total gastrectomy had been performed (by O.H.W.) for carcinoma of the stomach. In this operation the proximal end of the duodenum was closed and an anastomosis made between the esophagus and jejunum. As the removed specimen included the entire stomach, more than 2 cm. of esophagus and a portion of the duodenum, no doubt remains regarding the question of complete gastric excision.

Ten months following the procedure the patient returned for observation at our request, stating that he was in the best of health. X-ray examination demonstrated a good functioning stoma. There was no dilatation of the jejunum immediately distal to the anastomosis. Both vagi nerves were divided in the operative procedure but the patient's bowels moved regularly as previous to the operation. There was no evidence of anemia. He became hungry frequently and we felt that it would be of interest to investigate the hunger sensations in a gastrectomized patient.

Unfortunately he could not recall ever having had either before or after operation, any sensation he would describe as a hunger pang. To him the sensation was one of emptiness in the stomach followed by a feeling of general weakness. He was emphatic in stating that the sensation of hunger was now "just the same" as when he had a stomach. The only difference that he could notice was that he became hungry more frequently than before, which he

[1] Carlson, A. J., The Control of Hunger in Health and Disease. Univ. of Chicago Press. 1916.

[2] Ivy, A. C., and Vloedman, D. A., *Am. J. Physiol.*, 1925, **72**, 99.

attributed to the fact that he ate smaller quantities of food. Hunger was promptly relieved by eating a few crackers or drinking milk or buttermilk between meals.

In order to study the intestine for evidence of hunger contractions the patient was deprived of food and later instructed to swallow a small balloon which was fastened to the end of a rubber tube. The position of the balloon in the jejunum was verified by fluoroscopic examination. Tracings of the intestinal activity were recorded on a smoked drum through the medium of a water manometer. Each observation continued for about one hour.

Results. A tracing after 8 hours of starvation revealed practically no intestinal activity although the patient was very hungry.

He was starved for 24 hours but through an error was given a light breakfast 30 minutes before the tracing was obtained. Practically no intestinal activity was recorded. The patient stated that his hunger had been relieved.

Seven hours later when he had had only one light breakfast in a 32-hour period another tracing was taken. The patient was complaining of great hunger and weakness. The tracing revealed rather feeble rhythmical intestinal contractions of nearly uniform strength occurring 12 to 15 times a minute, but no hunger contractions comparable to those described for the stomach were observed. Respirations and body movements produced changes of pressure which were recorded on the drum, but these were satisfactorily differentiated from movements of the intestine itself. About 30 minutes after the introduction of the balloon the contractions became gradually weaker and finally ceased almost completely. This change followed the hypodermic injection of 5 units of insulin but probably was not due to the action of insulin. The patient stated at both the beginning and end of the experiment that he was experiencing hunger.

Although the hunger sensation described by this man is not characteristic of that said to be associated with gastric contractions it is a noteworthy fact that he was unable to detect any change in hunger sensations following complete gastric extirpation other than increased frequency of the hunger sensation. In this connection the observations of Ivy and Vloedman and of Quigley and Solomon[3] on the hunger contractions of the duodenum are of interest. The latter have reported that duodenal hunger contractions can occur in the absence of gastric motility. In the case reported here such a cause cannot be excluded; the activity of the small segment of

[3] Quigley, J. P., and Solomon, E. I., *Am. J. Physiol.*, 1930, **91**, 488.

jejunum investigated was not clearly related to the hunger which the man experienced. It is a significant fact, however, that patients with high intestinal fistula occasionally complain of constant hunger despite the frequent intake of food in liberal quantities.

The most important and definite observation brought out in the study of this patient is that a sensation interpreted by man as normal hunger which is relieved by the ingestion of food may occur following complete removal of the stomach.

5412

"True" Glucose Tolerance in Forty-two Normal Individuals.

G. WALTERMANN HOLT AND ESTHER M. GREISHEIMER.

From the Department of Physiology, the University of Minnesota.

Glucose tolerance in 18 women and 24 men, all in excellent health except 2, who were subjects of acute coryza, and who were students or faculty members of the University of Minnesota or physicians in practice in the Twin Cities, was investigated.

The subjects were fasted approximately 12 hours, beginning at 7 o'clock on the evening before the test. Blood samples were drawn at the end of the fast period, and then 50 gm. of glucose and the juice of one half lemon in 125 cc. of water were administered orally. Additional samples were drawn at the half hour, hour, and 2-hour periods, following the glucose. Incidental glycosuria was not investigated.

Protein removal was effected by the Somogyi zinc precipitation method.[1] Sugar determinations were made in duplicate by the improved copper-iodometric titration of the Shaffer-Hartmann method. This was demonstrated at the Thirteenth International Physiological Congress in Boston, in 1929, and directions were distributed at that time.

TABLE I.

Glucose Tolerance Data in Normal Individuals.

	Mean (mg. %)	Standard Deviation	Coefficient of Variation
Fasting	83.12±0.56	5.38±0.40	6.5
Half Hour	123.77±1.69	16.25±1.20	13.1
One Hour	109.10±2.39	22.99±1.69	21.1
Two Hours	72.37±1.71	16.44±1.21	22.7

[1] Somogyi, M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 353.

The data have been analyzed by the usual statistical methods. The means, standard deviations, and coefficients of variation are presented in Table I.

The means are shown graphically in the following curve:

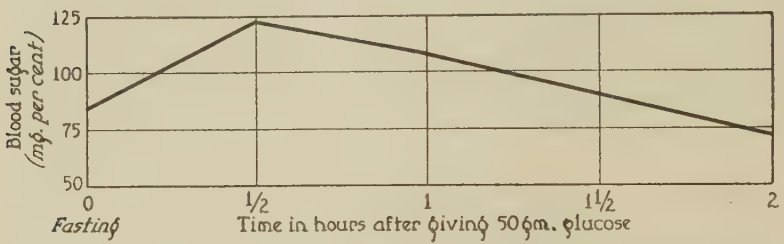

FIG. 1.

These data show a glucose value 15-20 mg. % lower than that obtained by other methods. Somogyi has shown that non-sugar reducing substances of the blood, which consist of glutathione, ergothioneine, and uric acid are not present in zinc filtrates.[1]

The highest glucose level obtains at the half-hour, the next highest at the hour, and at the end of 2 hours the glucose value is lower than the fasting value, showing that following stimulation of the pancreas the amount of insulin secretion is greater than the residual.

The authors are greatly indebted to all those who helped to make this investigation possible.

5413

Distribution of the Blood Sugar Between Plasma and Corpuscles in Man.

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In recent publications the suggestion that the blood sugar is unevenly distributed between corpuscles and plasma, and that the ratio of cell sugar to plasma sugar in blood of normal persons and of persons with diabetes is different, has been revived. Johns[1] has expressed the opinion that the corpuscles of the blood of persons with diabetes are less permeable to glucose than those of the blood of

[1] Johns, H. J., *J. Lab. and Clin. Med.*, 1930, **15**, 713.

normal persons. Folin and Svedberg,[2] on the other hand, have found evidence by the analysis of unlaked blood filtrates that the corpuscles of persons who have diabetes are slightly more permeable to glucose than the corpuscles of normal persons. The average ratios reported by Folin and Svedberg are 0.59 for normal persons and 0.69 for persons with diabetes, and are to be considered as representing the distribution of fermentable reducing substances. These values, indicating that the corpuscles contain less sugar than the plasma, are much lower than those obtained in a recent study in this laboratory, in which the well-known laked blood[3] filtrates were analyzed by the modified Shaffer-Hartman method.[4] With the object of investigating the rather large differences, a series of comparative analyses of the filtrates from laked and unlaked blood has been carried out.

The experimental procedure was designed particularly to avoid initial loss in the cell sugar by glycolysis and will be described in detail elsewhere. Unlaked blood filtrates were prepared with the mixed sodium sulfate-sodium tungstate reagent recently described by Folin.[5] Heparin was used as the anticoagulant. All results were corrected for nonfermentable reducing materials as determined by a modification of the technic of Somogyi,[6] and are reported here in terms of fermentable reducing substances. A modification of the Shaffer-Hartman reagent, a description of which has not been published, was used for the analyses. Blood was obtained from normal persons, either in the fasting state, or 2½ to 3 hours after breakfast.

In 14 experiments the distribution ratios calculated from the analysis of laked blood averaged 0.86. Unlaked blood filtrates prepared at the same time contained on the average 6 mg. for each 100 cc. of blood less fermentable reducing substances, corresponding to an average of 13 mg. for each 100 cc. less in the cells. The ratios of cell sugar to plasma sugar are thereby rather markedly depressed averaging 0.72.

A slight modification in the preparation of the unlaked blood filtrates has enabled us to obtain somewhat higher values, approximating closely the analyses of laked blood. This modification consists in cooling to 0°C. the sulfate-tungstate reagent into which the blood is measured for the extraction of sugar, and the maintenance of this low temperature during the period of extraction until the

[2] Folin, Otto, and Svedberg, Andrea, *J. Biol. Chem.*, 1930, **88**, 715.

[3] Folin, Otto, and Wu, Hsien, *J. Biol. Chem.*, 1919, **38**, 81.

[4] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

[5] Folin, Otto, *J. Biol. Chem*, 1930, **86**, 173.

[6] Somogyi, M., *J. Biol. Chem.*, 1928, **78**, 117.

addition of acid to complete precipitation of protein. The increase in the blood sugar in filtrates prepared by this device averaged about 4 mg. in each 100 cc. in 12 experiments. The differences, although negligible from the practical standpoint, and well within the ordinarily accepted limits of analytic error, are uniformly in one direction and indicate that the glucose content of our unlaked blood filtrates, as usually prepared, was too low. If the values for sugar in unlaked blood be increased by the amount indicated, the corrected distribution ratios will average about 0.80. This also represents approximately the ratio of the water content of the cells to that of the plasma, and suggests that the blood sugar tends to be distributed in equal concentrations in the water of the cells and of the plasma. The average distribution ratio found from the analyses of laked blood in these experiments, 0.86, is not in serious disagreement with this view, but may, however, imply that the cells tend to contain slightly more glucose relative to the plasma, rather than less, as found by Folin and Svedberg.

5414

Effect of Rattlesnake Venom on Flexner-Jobling's Carcinoma in the White Rat (Mus Norvegicus Albinus.)

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(Introduced by Frank C. Mann.)

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Since the venom of the rattlesnake is known to cause severe injury to normal animal tissues we were interested in determining its effect on the Flexner-Jobling carcinoma in the white rat. It was considered possible that intravenous injections of this venom would inhibit the development or possibly destroy the tumor in this animal. Therefore, the tumor was transplanted subcutaneously into 50 rats. Tumors subsequently developed in 29. Twelve of these were selected as controls, and 15 were selected for intravenous injections of the venom. The dose employed was 0.1 to 0.3 cc. of 2% crotalin, the average dose being about 0.2 cc. Larger doses proved fatal.

The injections were made once a week for 6 successive weeks and measurements were taken at the time of the injection. The results were entirely negative. The tumors of the injected rats grew just as rapidly as the control tumors.

It should be pointed out that the rat is highly refractive to the action of crotalin. A rat weighing 150 gm. is not killed by an injection of one-third of the usual lethal dose for a dog weighing 10 kg. A subcutaneous injection of crotalin into the white rat is not followed by sloughing which usually follows subcutaneous injections into dogs and rabbits.

5415

On the Cause of Brain Edema After Pitressin.

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Brain hydration has been shown[1] to accompany the anuria of rabbits injected with pitressin. We are now attempting by studies of brain swelling, to throw some light upon the cause of this pitressin edema.

In our first experiments, post-mortal swelling of dogs' brains in their own defibrinated blood was studied with and without the addition of pitressin. Pitressin (P. D. & Co.), which contains 0.5% chloretone was added, usually in the ratio of 2 cc. to each 25 cc. of defibrinated blood. In the control experiments, 2 cc. of 0.5% chloretone was added instead. (The brains were all obtained from dogs killed after 2 or 3 hours moderate etherization preceded by morphine.)

In each of 5 animals it was found that both the cerebrum and medulla swelled more slowly and to a less extent in the presence of pitressin. This difference was at first more pronounced in the cerebrum. The average weight increases from three nearly identical experiments for the second hour were: *cerebrum,* normal 7.1%, after pitressin 5.7%; *medulla,* normal 5.0%, after pitressin 4.5%. Four hours post mortem the following increases were noted: *cerebrum,* normal 7.8%, after pitressin 6.8%; *medulla,* normal 5.7%, after pitressin 4.5%.

Rabbits were given water *per os,* 75 cc. per kilo. Each day a rabbit was given, just after the water, ½ cc. pitressin per kilo. After an interval which varied from 15 minutes to one hour, the animals

[1] Ellerbrook, G. E., Dunham, E. S., and Barbour, H. G., *J. Pharmacol. and Exp. Therap.,* 1930, **39**, 249.

were killed by a blow on the head. In the blood of rabbits killed 15 to 30 minutes after pitressin injection, a normal rabbit brain gains considerably less weight than in its own blood. A pitressin rabbit's brain, however, shows in its own blood a normal degree of post mortal swelling. Thus the pitressin rabbit's blood appears to have the capacity to dehydrate a normal brain, just as when pitressin was added to the dog's blood in the *in vitro* experiments cited above. This point was tested on 6 pairs of rabbit cerebra and in 4 of these pairs we also tested the medullae. All these experiments gave the same qualitative result.

In the blood of rabbits killed 15 to 30 minutes after pitressin plus H_2O, normal rabbit brains showed the following respective weight increases during the first 3 hours: 2.5%, 5.0%, 6.6% (average of 6 experiments). The pitressin rabbits' brains in their own blood, gave the following: 4.5%, 6.7%, 8.1% (average of 6 experiments).

In the blood of rabbits killed 15 to 30 minutes after water only, the brains of the same rabbits gave the following: 4.4%, 6.1%, 7.8% (average of 6 experiments).

In 2 experiments in which the pitressin rabbit was killed respectively 45 and 60 minutes after the injection, a different picture was obtained. Here both the normal brain and the pitressin rabbit's brain were considerably *hydrated* by the pitressin rabbit's blood.

In the blood of rabbits killed 45 to 60 minutes after pitressin plus H_2O, normal rabbit brains showed the following respective weight increases during the first 3 hours: 6.7%, 9.2%, 9.5% (average of 2 experiments). The pitressin rabbits' brains, in their own blood, gave the following: 5.6%, 8.7%, 11.1% (average of 2 experiments).

In the blood of rabbits killed 45 to 60 minutes after water only, the brain of the same rabbit gave the following: 4.4%, 6.6%, 8.7% (average of 2 experiments).

The fact that the brain showed increased swelling in these latter cases may perhaps be best explained on the assumption that 45 minutes after injection significant amounts of pitressin will not remain in the blood. In the earlier paper we showed that rabbits given this dose of pitressin usually exhibit marked diuresis for the first half-hour, followed by anuria with brain edema. We find that the procedure described above produces more blood hydration in the pitressin rabbits than in the normals, the blood specific gravity continuing to fall for over one hour. In view of these several facts, we may attribute the later pitressin augmentation of brain swelling

as due to the contact of that organ with an abnormally hydrated blood from which the pitressin has largely disappeared.

Conclusions. 1. Pitressin, added to blood *in vivo* or *in vitro,* diminishes post-mortal swelling of the normal brain. 2. Pitressin probably disappears from the blood within 45 minutes after subcutaneous injection in rabbits, for brain swelling then becomes augmented. The brain edema previously demonstrated seems to result from the blood hydration which in turn is associated with anuria, and as previous experiments have shown, with loss of water from the skin.

5416

Guanidine Content of Blood from Epileptics.

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The guanidine content of blood from 18 epileptic men and 16 epileptic women, representing a total of 47 epileptic seizures, was determined by the nitroprusside method of Marston,[1] as modified by Weber,[2] and Major and Weber.[3] These blood samples were drawn near the termination of the seizures, often before the patient had completely recovered consciousness. As all of the epileptic subjects were institutional patients, blood samples from 9 non-epileptic men and 10 non-epileptic women, also inmates of the same institution, were studied by the same method to provide controls against the diet and routine institutional life of the epileptic patients. The blood guanidine data have been summarized in Table I.

From the table the high guanidine content of the blood from these epileptics in seizures is evident. The maximum blood guanidine found in the samples from the non-epileptic cases was 0.48 mg. per 100 cc. of blood, (individual data not presented in detail here), which value was exceeded in 29 of the 47 epileptic cases. The average blood guanidine for the non-epileptics was near 0.2 mg. per 100 cc., which is slightly higher than the average from the non-hypertension cases presented by Major and Weber[3]

[1] Marston, Austral, *J. Exp. Biol. Med. Sci.,* 1924, **1**, 99.

[2] Weber, PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 712.

[3] Major and Weber, *Arch. Int. Med.,* 1927, **40**, 891.

TABLE I.

Guanidine mg. per 100 cc.	Blood samples from epileptics in seizure			Blood samples from non-epileptics		
	Men	Women	Total	Men	Women	Total
0		1	1	1	6	7
.09	2	1	3		1	1
0.1—0.19	1		1	2	1	3
0.2—0.29	2		2	1	1	2
0.3—0.39	6	3	9	3		3
0.4—0.49	3		3	2	1	3
0.5—0.59	8		8			
0.6—0.69	3		3			
0.7—0.79	5	1	6			
0.8—0.89		2	2			
0.9—0.99	1		1			
1.0—1.09		1	1			
1.1—1.19		3	3			
1.2—1.29		2	2			
2.15		1	1			
2.22		1	1			
No. of cases	31	16	47	9	10	19

but is much the same as the normals given by Traut and MacFate.[4] Regardless of the actual value of normal blood guanidine, all but 4 of the 47 epileptic cases had higher blood guanidine than the average of 0.2 mg. per 100 cc. of the comparable non-epileptic cases from the same institution.

While it is not possible to go into the clinical histories of these cases here, neither hypertension nor nephritic conditions, which have been correlated by Major and Weber[3] in certain subjects with high blood guanidine, were found in any of the epileptic subjects reported here.

The finding of high blood guanidine near the end of the epileptic seizures does not, of course, give guanidine a causal rôle in epilepsy, but it is suggestive in view of the guanidine findings in parathyroprivia tetany reported by Paton[5] and Ellis,[6] and more recently in general tetany by Traut and MacFate.[4]

[4] Traut and MacFate, *J. Am. Med. Assn.*, 1931, **96**, 266.

[5] Paton, *et al.*, *Quart. J. Exp. Physiol.*, 1916, **10**, 315.

[6] Ellis, *Biochem. J.*, 1928, **22**, 931.

5417

Chylomicron Content and Total Lipids of Blood Plasma as Determined on Dogs.

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The method described by Gage and Fish[1] of studying the visible lipemia of the blood by means of the dark-field microscope is simple and requires only 1 drop of blood and a few minutes to count the chylomicrons. By using a net micrometer in the eyepiece of the microscope and by counting very rapidly the chylomicrons in each small square, a fairly accurate count is made. An accuracy within the limits of experimental error in many physical measurements is obtained.

Since the chylomicrons have been shown to be dependent on the fatty portion of the diet, it was thought that the determination of the chylomicrons by the dark-field microscope might give a measure of the total amount of fat present. It was the purpose of the present investigation to find out if the chylomicrons measure the total amount of fat present or what the relationship may be between the fat in the blood determined by chemical means and the chylomicrons.

In the present study in which dogs were used, blood was taken from the heart, in order to obtain a 15 cc. sample.

The method of feeding used for previous determinations[2] was followed. The dogs were fasted overnight, about 20 hours. A 15 cc. sample of blood was taken from the heart, the chylomicrons counted in a sample of the blood and the remainder used for analysis[3] to find the total lipids in the blood. The animals were then fed 40 gm. of butter and every 2 hrs, thereafter 15 cc. of blood were taken. It is the opinion of the writer that when more than 40 gm. of a fat are fed, it becomes too difficult to count the chylomicrons with any degree of accuracy.

Several mongrel dogs, weighing between 11 and 19 kg. were used. The history of the dogs, their age and diet previous to the time they came to laboratory are not known. But for at least one week before the experiment, they were fed on dog biscuit from the Kennel Food Supply Co., and water. A summary of the results for each hour the samples were taken is given in Table I.

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[1] Gage, S. H., and Fish, P. A., *Am. J. Anat.*, 1924, **34**, 1.

[2] MacArthur, E. H., *J. Biol. Chem.*, 1930, **87**, 299.

[3] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

TABLE I.
Average Data Obtained for Digestive Cycle of 4 Dogs Fed Butter.

Average Weight in kg.	Sample Taken	Blood		Coefficient Correlation
		Total Lipids mg. per 100 cc.	Chylomicrons	
13	Before feeding fat	741	54	0.46±0.24
	2 hours after	778	67	
	4 " "	792	71	
	6 " "	665	70	
	8 " "	830	61	

In the morning before food is taken the chylomicron count is higher in the dogs used than with normal human beings. The total lipids in mg. per 100 cc. of plasma, however, differ little from results on fasting dogs of Terroine.[4]

A considerable variability among the dogs was found with not a high correlation between the chylomicron counts and the total lipids of the blood. Terroine[4] also, reported great variation among dogs, due, as he believed, to the fat reserves of the different animals. But Bloor,[5] by means of the nephelometric method, reported the percentage of fat in the blood to be fairly constant. He said also, that fasting may or may not produce an increase in blood fat depending apparently on the "nutritional condition of the animal." An animal which did not ordinarily show an increase of blood fat in fasting, he said, showed the usual curve of increase of blood fat in fasting, after being stuffed with fat food for a week before the fast. This would indicate that we might expect variations due to the fat reserves of the different animals, as Terroine showed.

Knudson and Grigg[6] reported 2 experiments with dogs that were fed 70 cc. of olive oil. Chylomicron counts and the determination of total cholesterol and total fatty acids of the blood were made. Coefficients of correlation between the chylomicrons and the total lipids of each of their experiments were 0.36±0.26 and 0.75±0.13, respectively. From these determinations they concluded that the fat in the blood is not all in chylomicron form.

Although the present investigation does not show whether or not the fat in the blood is all in chylomicron form, it would appear from the results that a greater relation exists between the total lipids in the blood and the chylomicrons than has been shown before.

[4] Terroine, E., Theses, 1919.

[5] Bloor, W. R., *J. Biol. Chem.*, 1914, **19**, 1.

[6] Knudson, A., and Grigg, W. K., PROC. SOC. EXP. BIOL. AND MED., 1923, **20**, 462.

A comparison of the fat in the blood found by the chylomicron count, using the dark-field microscope, with the fat found by chemical analysis was made, using dogs as the experimental animals. Under the conditions of the experiment there appears a correlation, although not high, between the chylomicron content of the blood and the total lipids of the blood found by chemical analysis.

The writer is indebted to Dr. J. A. Dye for the use of the physiology laboratory and dogs in this study.